2ND EDITION

ESSENTIALS OF VETERINARY PHARMACOLOGY AND THERAPEUTICS

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CHAPTER 1 INTRODUCTION

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Etymologically, pharmacology is the science of drugs (Greek: *pharmakon* = drug or remedy, *logos* = study). In actual scientific use, however, its meaning is limited to the study of the actions of drugs on living systems. In broader sense, Pharmacology as a science deals with all aspects of knowledge about drugs including sources, drug-development, physical and chemical properties, biochemical and physiological effects, mechanism of action, absorption, distribution, biotransformation, excretion, therapeutic and other uses, and adverse effects. The pharmacology has great importance both from the professional and economic points of view. It is impossible to think of medical and veterinary medicines not connected to drugs, as drugs have formed an integral part of treatment from the beginning of human civilization.

DEFINITIONS AND TERMINOLOGY

1. Sub-Divisions/Branches of Pharmacology

Pharmacology has following major sub-disciplines/branches:

a. **Chemotherapy:** It deals with the study of drugs which selectively inhibit or destroy specific agents of diseases such as bacteria, viruses, fungi and parasites.

b. **Clinical pharmacology:** It deals with the study of drugs in man and animals as pertaining to or founded on clinical observations (study does not include theoretical or experimental knowledge).

c. **Experimental pharmacology:** It deals with the study of action of drugs in animals/animal tissues under laboratory conditions.

d. **Medicinal chemistry:** It deals with the study of designing and synthesis of new drugs. It is largely based on the structure activity relationship data of existing drugs belonging to one generic group.

e. **Pharmacoeconomics:** It deals with the study of the cost-effectiveness of drug treatments.

f. **Pharmacoepidemiology:** It deals with the study of both beneficial and adverse effects of a drug on human/animal population.

g. **Pharmacodynamics** (Greek: *dynamis* = force): It deals with the study of biochemical and physiological effects of drugs and their mechanism of action. In other words, it means "What the drug does to the body".

h. **Pharmacogenetics/Pharmacogenomics:** It deals with the study of genetically determined variations in response to drugs.

i. **Pharmacognosy** (Greek: *gnosis* = knowledge): It deals with the study of drugs derived from plant and animal sources (their sources, identification, constituents and detection of substituents or adulterants).

j. **Pharmacokinetics** (Greek: *kinesis* = movement): It deals with the study of absorption, distribution, metabolism and excretion (ADME) of drugs. In other words, it means "What the body does to the drug".

k. **Pharmacometrics:** It deals with the study of qualitative and quantitative evaluations of the activity of drugs.

l. **Pharmacotherapeutics** (Greek: *therapia* = medical treatment): It deals with the use of drugs in the prevention and treatment of diseases. In other words, it is the clinical application of the pharmacokinetic and pharmacodynamic knowledge of the drug.

m. **Pharmacy**: It deals with the study of collection, compounding and dispensing of drugs so as to make them fit for administration to patients.

n. **Toxicology** (Greek: *toxicon* = poison): It deals with the study of adverse effects of drugs or chemicals on living systems.

2. Therapeutics

Therapeutics (Greek: *therapeutike* = medical practice) is a practical branch of medicine dealing with the application of all sciences in the treatment of diseases. It includes use of drugs, surgery, radiation, diets, exercise and management practices. Therapeutics consists of following types of therapies (treatments):

a. **Empirical therapy:** It is the application of drugs not derived from the scientific method, but on mere experience

gained by the clinician. It is occasionally used when the knowledge of either nature of disease or action of drug is not known, but the therapy has been found effective e.g. use of sodium chloride in piroplasmosis and chloral hydrate in acetonaemia in cattle. In the medical profession, the term is also used when treatment is started before a diagnosis is confirmed e.g. use of antibiotics before culture sensitivity report arrives.

b. **Rational therapy or specific therapy:** It is the application of drugs based on the scientific knowledge of pharmacology of the drug and on pathology of the disease. Therefore, it is a treatment based on principles when the nature of disease as well as action of drug is well understood. For example, use of atropine in organophosphorus insecticides poisoning and sodium bicarbonate in hyperacidity of the stomach.

c. **Symptomatic therapy:** It is the application of drugs for the treatment of only symptoms with or without a proper diagnosis. The symptomatic treatment without a proper diagnosis is not advisable, yet it is practiced occasionally to save animals from the immediate consequences of the harmful symptoms. For example, use of CNS depressants (e.g. pentobarbital or diazepam) to control convulsions induced by an unknown toxicant.

d. **Palliative therapy:** It is the application of drugs or any other form of medical care to control the severity of disease symptoms, rather than striving to halt, delay, or reverse progression of the disease itself or provide a cure. The primary aim of palliative treatment is to prevent and relieve suffering and to improve quality of life for patients facing serious, complex illness such as cancer. Palliative therapy is often included in the symptomatic therapy.

e. **Supportive therapy:** It is the application of drugs directed towards maintaining the patient's physiological or functional integrity until more definitive treatment can be carried out, or until the patient's recuperative powers function to obviate the need for further treatment. Many drugs can provide supportive therapy and in many cases it forms the major portion of therapeutics e.g. use of parenteral fluids in dehydration.

f. **Substitutive or replacement therapy:** It is the application of drugs directed towards supplying an endogenous substance, which is normally present in body but becomes deficient in a patient because of disease, injury, congenital deficiencies, etc. For example, adrenocortical hormones are used in the treatment of a patient with Addison's disease as substitutive therapy.

g. **General therapy or Accessory therapy:** It is the treatment of diseases without use of drugs. It may be classified as follows:

   i. *Mechanical therapy:* It is the use of exercise, massage and pressure in the treatment of diseases.

   ii. *Physical therapy:* It is the use of light, heat or other physical agents in the treatment of diseases.

   iii. *Heliotherapy:* It is the use of sunlight in the treatment of diseases.

   iv. *Hydrotherapy:* It is the use of water in all forms in the treatment of diseases.

   v. *Dietic therapy:* It is the regulation of diet in the treatment of diseases or abnormal conditions e.g. restriction of sugar in the management of obesity and diabetes.

   vi. *Psychotherapy:* It is the use of hypnotism and advises in the treatment of human patients suffering from mental diseases.

   vii. *Placebo therapy:* It is the use of inert harmless substances like calcium lactate or dextrose simply to satisfy the owner/patient. It is mostly used in human medicine for psychological or psycho-physiological effects on the patients.

3. Pharmacy and Related Terms

a. **Official pharmacy:** It is the preparation and compounding of drugs according to the official pharmacopoeia.

b. **Extemporaneous pharmacy:** It is the preparation and compounding of prescription of a qualified practitioner. It is based mainly on the personal experience of the practitioner.

c. **Galenical:** It is the preparation of drugs which are sanctioned by the pharmacopoeia and is often used in the same sense as 'official'. It generally refers to the preparations from crude drugs such as tinctures, extracts, etc.

d. **Compounding:** It is the method by which a drug is prepared.

e. **Dispensing:** It is the art of labelling and dispatching of medicinal preparations generally for immediate use of patients.

f. **Pharmaceutics:** It is the science dealing with preparation of drugs/medicinal preparations and generally refers to large-scale manufacturing of drugs.

g. **Biopharmaceutics:** It deals with the development of new drug delivery systems and new dosage forms. It also provides information how these dosage forms can influence the pharmacodynamic and pharmacokinetic properties of a drug.

h. **Posology** (Greek: *posos* = how much): It deals with study of determination of drug dosage of remedies. It may also be defined as the study of drug dosage.

i. **Metrology:** It deals with the study of weights and measures as applied to preparation and administration of drugs.

j. **Materia medica** (Latin term meaning medical materials): It is an obsolete purely descriptive term/subject that was concerned with pharmacy, pharmacognosy, posology and indications for therapeutic uses of drug. The term was used from the period of the Roman Empire until the twentieth century, but has now been generally replaced in medical education by the modern term pharmacology.

4. Drug, Dose and Related Terms

a. **Drug** (French: *drogue* = dry herb): Drug may be defined in different ways such as:

   Drug is a chemical agent used for the diagnosis, prevention, mitigation (to lessen severity or sufferings) or treatment of a disease.

Drug is a single chemical agent, other than food, which modifies functions of living system. In broad sense, all biologically active chemicals including therapeutic agents, poisons and addictive substances are called drugs.

WHO (1966) definition of a drug is any substance or product that is used or intended to be used to modify or to explore physiological system or pathological states for the benefit of the recipient.

b. **Therapeutic agent:** It is an agent or a substance which is capable of producing a curative effect in a disease state. This may include pharmaceuticals (Preparation of drugs), nutraceuticals (Nutritional supplements, functional/designer foods) and biologicals (Vaccines, bacterins, toxoids, antiserums and antitoxins).

c. **Medicine:** It is a trivial term used for a chemical agent or a mixture of chemical agents, often combined with pharmacologically inactive substance(s), used for prevention, mitigation or treatment of a disease (unlike drug, the term medicine is not used for poisons or addictive agents).

d. **Xenobiotic:** It is any chemical substance that is not nutrient for body (foreign to body) and which enters the body through ingestion, inhalation or dermal exposure. The term includes drugs, industrial chemicals, pesticides, pollutants, plant and animal toxins, etc.

e. **Ligand:** A ligand is a specific substance that is able to bind to and form a complex with a biomolecule to serve a biological purpose. The ligand may include a signal triggering molecule such as a hormone, neurotransmitter or drug binding to the active site on a receptor.

f. **Pro-drug:** It an inert drug precursor, which upon biotransformation liberates the pharmacologically active parent compound.

g. **Pharmacological tool:** It is a pharmacologically active chemical or drug used for experimental purposes.

h. **Drug (Substance) of abuse:** It is a chemical or therapeutic agent used for other than therapeutic purposes.

i. **Over-the-counter (OTC) drug:** It is a drug or a pharmaceutical product that may be sold directly to a consumer without a prescription from a health care professional e.g. aspirin, sunscreens, anti-dandruff shampoos, etc.

j. **Prescription drug:** It is a drug that is sold only on the presentation of a prescription from a registered practitioner. The prescription drugs are sold only by registered drug stores or pharmacies and, unlike OTC drugs, are not freely available.

k. **Dosage form:** It a drug preparation that provides convenient mode of administration of a drug to the patient.

l. **Dose:** It is the total (absolute) quantity or amount of a drug applied or administered all at one time to a patient to get the desired pharmacological/therapeutic response.

m. **Dosage:** It the application or administration of a drug in prescribed amounts to a patient to get desired pharmacological/therapeutic response (dosage usually indicates the dose, frequency and duration of therapy e.g. 100 mg b.i.d. x 4 days).

n. **Dosage regimen:** It refers to the complete schedule of drug therapy including the dose, route of administration, frequency of administration and duration of therapy (e.g. 100 mg, IM, b.i.d x 4 days). For practical purposes, the terms dosage and dosage regimen are used interchangeably.

o. **Dose rate:** It is the expression of dose in terms of amount of drug per unit body weight (mg/kg or μg/kg) or for certain purposes per unit surface area (mg/m^2).

p. **Initial dose/Loading dose:** It is a relatively large dose of a drug given at beginning of therapy to get the desired pharmacological effect at the earliest.

q. **Maintenance dose:** It a dose(s) given during the course of therapy to maintain desired pharmacological effect produced by the initial dose.

r. **Divided dose/Fractional dose:** It is the definite fraction of a full dose given repeatedly at short intervals so that full dose is administered within a specified period, usually one day.

s. **Lethal dose:** It is a dose of a chemical or drug that is likely to cause death.

t. **Standard dose:** It is a dose that is suitable for a large number of animals in a population i.e. individual variations are minor.

u. **Regulated dose:** It is a dose that is accurately adjusted by repeated measurement of the affected physiological parameters e.g. antihypertensives and general anaesthetics.

v. **Titrated dose:** It is a dose of a potent drug that is arrived at by titrating it with acceptable degree of adverse effects. Low initial dose and upward titration (in most non-critical situations) or high initial dose and downward titration (in critical situations) can be practiced.

5. Effects/Activity and Miscellaneous Terms

a. **Pharmacological effect:** It is an effect(s) produced by the pharmacological actions of a therapeutic dose of a drug in the body.

b. **Toxic/Adverse effect:** It is an undesirable effect produced by a drug that is detrimental to either survival or normal functioning of the animal.

c. **Side effect:** It is an undesirable effect produced by normal pharmacological actions of a drug, which may not be detrimental or harmful to the animal.

d. **Transient effect:** It a pharmacological effect produced by a drug that remains for short duration of time.

e. **Latent effect:** It is a pharmacological effect produced by a drug that has latent onset of action.

f. **Cumulative effect:** It is a progressive pharmacological effect produced by summation of incremental doses resulting from successive exposures to a drug.

g. **Withdrawal effect:** It is an effect produced by abrupt discontinuance of a drug, especially an addicting agent, after its long-term treatment.

h. **Risk-Benefit ratio:** The phrase 'risk-benefit ratio' is used to describe the adverse effects of a drug in relation to its beneficial effects. In therapeutics, the beneficial effects

(e.g. effectiveness of drug, reduced morbidity and ease of drug administration) should outweigh the adverse effects (e.g. toxicity, cost and inconvenience of administration).

i. **Structure-activity relationship (SAR):** The phrase 'structure activity relationship' is used to describe the relationship between the structure of a drug and its pharmacological activity. It primarily deals with the study of how chemical alterations to a basic chemical pharmacophore (the basic structure which gives a pharmacological effect) could alter the action of a drug.

DRUG INFORMATION SOURCES

There are many reliable sources of drug information, which provide authenticated knowledge about established drugs and furnish information for understanding newer ones. These include pharmacopoeias, formularies, drug reference books, pharmacology textbooks, journals, encyclopaedias and drug database websites. Then there are other sources of drug information, which may provide details such as trade names, formulations, dosage forms, methods of storage and dosage range for therapeutic use necessary mainly from marketing and clinical use point of view.

I. Pharmacopoeias

The pharmacopoeias (Greek: *pharmakon* = drug, *poieo* = to nake) are official books on drugs containing a selected list of the stablished drugs and medicinal preparations with descriptions of heir physical and chemical properties, tests for their identification, urity, potency, minimum standard required and average dose for dults. The pharmacopoeias are published under the authority of recognised body, generally constituted by law, to ensure niformity in composition and strength of medicines used in the eatment of diseases. Drugs included in pharmacopoeias are alled 'official drugs', while other drugs are called 'non-official ugs'. Many countries have their own pharmacopoeias.

a. **Indian Pharmacopoeia (I.P.):** The Indian Pharmacopoeia is the legally recognised drug compendium for the quality of drug substances and preparations and other health care products manufactured or sold in India. It is published by the Indian Pharmacopoeia Commission, which is an Autonomous Institution under the Ministry of Health & Family Welfare, Government of India. The first edition of the Indian Pharmacopoeia was published in 1955 and subsequent editions were published in 1966, 1985, 1996, 2007 and 2010. The sixth edition of the Indian Pharmacopoeia entitled 'Indian Pharmacopoeia 2010' is presented in three volumes with total 1918 monographs, out of which 287 are added first time. Addendum 2012 to IP 2010 has also been released and became effective from 1st January, 2012. Standards for veterinary drugs and products which were published as a supplement to the previous editions of the Indian pharmacopoeia now form an integral part of this compendium.

b. **British Pharmacopoeia (B.P.):** The British Pharmacopoeia is produced by the British Pharmacopoeia Secretariat of the Medicines and Healthcare products Regulatory Agency. The first official British Pharmacopoeia was published in 1864 and since then it has been providing authoritative official standards for pharmaceutical substances and medicinal products in United Kingdom and more than 100 countries. Now it is annually updated and is used by individuals and organizations involved in pharmaceutical research, development, and manufacturing and testing. The BP 2013 comprises of five volumes of the British Pharmacopoeia 2013 and a single volume of the British Pharmacopoeia (Veterinary) 2013, along with a fully searchable CD-ROM and online access. The British Pharmacopoeia maintains close ties with the work of the European Pharmacopoeia and also includes monographs of the European Pharmacopoeia.

c. **United States Pharmacopoeia (U.S.P.):** The United States Pharmacopeia (USP) is the legally recognised book of standards for the quality of drug substances and preparations included therein for the U.S.A. and most Central and South American Republics. It is published by the United States Pharmacopoeial Convention, Rockville, Maryland. The first edition of the USP was published in 1820. It is now published in combination with the National Formulary as United States Pharmacopeia-National Formulary (USP–NF). The USP contains legally recognized standards of identity, strength, quality, purity, packaging and labelling for drug substances, dosage forms and other therapeutic products, whereas the NF contains standards for products such as excipients. The 2013 Edition of the United States Pharmacopeia and National Formulary (USP 36 – NF 31) is the latest edition of US official drug compendium.

d. **European Pharmacopoeia (Ph. Eur.):** The European Pharmacopoeia (*Ph. Eur.)* is the official drug compendium in about 37 countries including the members of the European Union. Some countries (e.g. United Kingdom and Germany) have their additional local pharmacopoeias. The European Pharmacopoeia is developed by the European Directorate for the Quality of Medicines (EDQM) and contains a wide range of active substances and excipients used to prepare pharmaceutical products in Europe. The first edition of *Ph. Eur.* was published in 1964 and since 2005 the pharmacopoeia is published in 2 volumes. Volume 1 contains general chapters and monographs (e.g. on dosage forms, methods of analysis, reagents) and volume 2 contains all substance monographs. The monographs give quality standards for all the main medicines used in Europe. The latest 7th edition of European Pharmacopoeia consisting of two initial volumes (Vol. 1 and 2) and two supplements (Supplement 7.1 and 7.2) was published in 2010 and became effective from 1 January 2011.

e. **International Pharmacopoeia (Ph.Int.):** The International Pharmacopoeia (Ph.Int.) is a publication of the World Health Organization and constitutes a collection of recommended procedures for analysis and specifications for the determination of pharmaceutical substances, excipients and dosage forms which are intended to serve as source material for reference or adaptation by any WHO Member State. It also provides recommended standards for international use and is of particular value to developing countries where it is a basis of drug control

and establishment of National Pharmacopoeias. Priority is given to medicines those are widely used throughout the world, particularly to drugs which are important to WHO health programs. The first International Pharmacopoeia was published by the WHO in 1951 (volume I) and in 1955 (volume II). The present Fourth Edition of International Pharmacopoeia comprises of Volumes 1 and 2, published in 2006, and amended and augmented by the First Supplement published in 2008 and the Second Supplement published in 2011.

2. Formularies

Formularies are lists of prescription drugs or medicines, both generic and brand names, to which prescribers are encouraged or required to adhere, depending on the type of health plan being considered. The main reason for developing formularies is to promote rational prescribing and to limit costs. The formularies are developed by pharmacy and therapeutics committees composed of pharmacists and physicians from various medical specialties and are revised periodically like pharmacopoeias. Some countries have their own Pharmacopoeias and official National Formularies e.g. British National Formulary (BNF) and United States Pharmacopeia-National Formulary (USP–NF). Some hospitals maintain their own special Formularies and handbooks concerning the drugs and formulations used frequently in the Institutions and encourage hospital doctors to prescribe from the selection of drugs contained therein. Formularies are smaller in size and much handier compared to the pharmacopoeias.

a. **British National Formulary (B.N.F.):** The British National Formulary is a joint publication of the British Medical Association and the Royal Pharmaceutical Society of Great Britain. It contains classified notes on drugs and preparations used in the treatment of diseases. The BNF provides UK healthcare professionals with authoritative and practical information on the selection and clinical use of medicines in a clear, concise and accessible manner. It is published twice a year (in March and September) and the September 2012 issue (British National Formulary 64) is the current edition.

b. **Veterinary Formulary:** The Veterinary Formulary is a publication of the Royal Pharmaceutical Society and British Veterinary Association. The first edition of the Veterinary Formulary appeared in 1991 and subsequent editions were published in 1994, 1996, 1998, 2001 and 2005. It serves the purpose of a rapid reference text for veterinarian practitioners, veterinary students and others involved with animal health care. It is also useful to academicians, pharmacists and researchers for a quick reference.

c. **National Formulary (N.F.):** It is issued by the American Pharmaceutical Association to provide standards and specifications for maintaining the quality of pharmaceuticals and therapeutic agents. The first edition of the "National Formulary" (originally named "The National Formulary of Unofficial Preparations") came out in 1888. Now it is published in combination with the U.S. Pharmacopoeia.

d. **National Formulary of India (NFI):** The National Formulary of India (NFI) is a publication that contains guidelines on right dosage of medicines for drug prescribers. It is essentially meant for the guidance of the members of the medical profession: medical students, nurses and pharmacists working in hospitals and in sales establishments. The NFI was first published in India in 1960 with subsequent two more editions in 1966 and 1979. The fourth edition of National Formulary of India 2011, the publication prepared by the Indian Pharmacopoeia Commission (IPC), has been published after a gap of 3 decades.

3. Other Sources

Numerous other sources provide reliable information on drugs. These include reference books and publications of various associations, societies, non-governmental bodies, pharmaceutical companies, hospitals, etc.

a. **Martindale: The Complete Drug Reference:** It is a reference book on drugs and medicaments currently in use throughout the world. It was earlier published as Martindale: The Extra Pharmacopoeia, the first edition of which was compiled and published by William Martindale in 1883 and is still known as Martindale by its users. The 37th edition of Martindale: The Complete Drug Reference, published in 2011, contains up to date information about 6,000 drug monographs, 161,000 preparations 54,000 reference citations and 15,000 manufacturers with coverage of proprietary preparations expanded to 41 countries and regions. Martindale contains information on drugs in clinical use worldwide, as well as selected investigational and veterinary drugs, herbal medicines, pharmaceutical excipients, vitamins and nutritional agents, vaccines, diagnostic agents, drugs of abuse, toxic substances, disinfectants and pesticides. This compendium contains references to some drugs which could not be found anywhere else, in particular drugs which are no longer in common use or those have toxicity levels normally unacceptable in therapeutics.

b. **Merck Index:** The Merck Index is an encyclopaedia of chemicals, drugs and biologicals published by the United States pharmaceutical company Merck & Co. It was first published in 1889. The current 14th edition, released in 2006, contains more than 10,000 monographs, 27 supplemental tables and 450 organic name reactions. The Merck Index serves the purpose of scientists and practitioners by providing comprehensive accurate information on drugs and chemicals.

c. **Monthly Index of Medical Specialties (MIMS):** The Monthly Index of Medical Specialties (MIMS) is a pharmaceutical prescribing reference guide published in the United Kingdom since 1957 by Haymarket Group, the largest privately-owned publishing company in the UK, and lists preparations about all drugs in the UK formulary. MIMS is also published by unrelated organizations in several other countries including India. MIMS INDIA, launched in 1980, is published every month by M/S. A.E. Morgan Publications (India) Private Limited, New Delhi and provides up-to-date information on all major drug preparations available for prescription in India. It is primarily designed as a reference and prescribing guide

for registered medical practitioners and pharmacists and is India's most widely used medical journal.

d. **Current Index of Medical Specialties (CIMS, INDIA):** It is a commercial, Indian drug index published quarterly by M/S Biogard Private, Ltd., Bangalore and serves the same purpose as mentioned under MIMS, INDIA.

HISTORY

Pharmacology in the present form is a relatively recent branch of medical science, about a hundred years old. But it existed in one or the other form since the dawn of civilization. Throughout recorded history, mankind has employed drug substances for treating diseases as well as for social and religious purposes. Some of the important historical developments and personalities are given below.

1. Ancient Medicine

a. **Ayurveda:** Ayurveda (Sanskrit: *ayus = life, veda =* science) is a system of traditional medicine native to the Indian Subcontinent. The earliest literature of Ayurveda appeared during the Vedic period in India between 4000 and 600 BC and deals mainly with different aspects of science of life and art of healing. Ayurvedic practitioners identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases. Today, Ayurveda is considered to be a form of complementary and alternative medicine in the world, where several of its methods, such as the use of herbs, massage and Yoga as exercise are applied in the prevention and treatment of diseases.

b. **Kahun Papyrus (1800 BC):** The Kahun Papyrus is as an ancient Egyptian text discussing various mathematical and medical topics. Its many fragments were discovered at town of El-Lahun (Kahun) by Flinders Petrie in 1889 and are kept at the University College London. The Kahun Medical Papyrus (Kahun Gynaecological Papyrus) is considered to be the oldest known medical text of any kind, dated to about 1800 BC. It documented mainly the women's problems - gynaecological diseases, fertility, pregnancy, contraception, etc. with their diagnosis and treatment. Some portion of the text also deals with veterinary medicine.

c. **Ebers Papyrus (1550 BC):** Ebers Papyrus (also Papyrus Ebers) is a famous ancient Egyptian document written about 1550 BC. It was found in a tomb of a mummy in Thebes and is now preserved at the University of Leipzig, Germany. It contains information about a number of diseases and some 700 formulas and remedies. This included chapters on contraception, diagnosis of pregnancy and other gynaecological matters, intestinal diseases and parasites, eye and skin problems, dentistry and surgical treatment of abscesses and tumours, bone-setting and burns. Various prescriptions including those of castor oil, opium, colchicum and some other drugs are still in use.

d. **Pen Tsao:** Pen Tsao (originally called *Shen Nung Pen Ts'ao Ching)* is the great herbal 'Materia Medica' written in China between the third and fifth centuries AD. It documented the work of Emperor Shen Nung and his followers, who lived in about 2700 BC. It recorded over 365 medicinal substances including many vegetable and mineral preparations and a few animal products as well, some of which are applicable even today.

e. **Hippocrates (460-370 BC):** Hippocrates, a Greek physician, is considered as 'Father of Medicine' in recognition of his enormous contributions to the science as the founder of the Hippocratic School of medicine. He was the first person to recognise disease as an abnormal reaction of the body rather than as a visitation from the Gods. He tried to organise the science of medicine on the basis of observations, analysis and balancing the body humours and categorize illnesses as acute, chronic, endemic and epidemic. He introduced the use of metallic salts for the treatment of a variety of body disorders.

f. **Theophrastus (371-287 BC):** Theopharastus, a great Greek philosopher and natural scientist, is called 'Father of Pharmacognosy'. His interests were wide-ranging, extending from biology and physics to ethics and metaphysics. The most important of his books are two large botanical treatises, *Enquiry into Plants* and *On the Causes of Plants*, which constitute the most important contribution to botanical science during antiquity and the Middle Ages. He systematically classified medicinal plants on the basis of their medicinal characteristics.

g. **Pedanius Dioscorides (30-90 AD):** Pedanius Dioscorides was an ancient Greek physician and botanist, who practiced during the time of Roman emperor Nero. In 70 AD, he compiled a five-volume book in his native Greek, known in Latin as *De Materia Medica.* This five book study focused upon "the preparation, properties and testing of drugs", which served as a precursor to all modern pharmacopoeias, and is one of the most influential herbal books in history. It describes about 600 plants of medicinal value and provides knowledge about the herbs and remedies used by the Greeks, Romans, and other cultures of antiquity. Dioscorides also classified poisons according their origin like animal, vegetable and mineral poisons.

h. **Galen (131-200 AD):** Claudius Galenus, better known as Galen, was a prominent Roman physician and philosopher of Greek origin. Galen earned a reputation as a practitioner and a public demonstrator of anatomy as he performed extensive dissections and vivisections on animals. He described many formulae containing plant and animal drugs. He compiled this knowledge in 20 books called as Galenical Work. His theories dominated and influenced the medical science for well over a millennium.

i. **Muhammad ibn Zakariya al-Razi (865-925 AD):** Muhammad ibn Zakariya al-Razi, known as Rhazes, was a Persian physician and philosopher who made numerous advances in medicine through own observations and discoveries. He was the first physician to distinguish smallpox and measles through his clinical characterization of the two diseases. He was also the first physician who systematically used alcohol in his clinical practice. Zakariya al-Razi contributed in many ways to the early practice of pharmacy by introducing the use of 'mercurial

ointments' and his development of apparatus such as mortars, flasks, spatulas and phials, which were used in pharmacies until the recent times. Zakariya al-Razi recorded his own clinical experiences and observations in a medical encyclopaedia comprising of nine volumes called "al-Hawi" in Arabic (called *The Large Comprehensive* or *Continens Liber* in Europe).

j. **Paracelsus (1493-1541 AD)**: Paracelsus is regarded as the first systematic botanist and is also credited for giving zinc its name, calling it *zincum*. He pioneered the use of chemicals and minerals for treating diseases. He introduced mercury in the treatment of syphilis and distilled oils in medicine. Paracelsus was also responsible for the creation of laudanum, an opium tincture very common until the 19th century. He gave the great statement "All substances are poisons; there is none which is not poison. The right dose differentiates a poison and a remedy".

k. **Valerius Cordus (1514-1544 AD):** Valerius Cordus was a German physician and botanist who compiled the first pharmacopoeia and carefully described techniques to be employed in preparation of drugs. He is also widely credited with having pioneered a method for synthesizing ether. Cordus also identified and described several new plant species. His free thoughts and critical inquiry were in contrast to the secrecy prevailing prior to his time.

2. Modern Pharmacology

Conversion of the old medicine into the modern pharmacology is fairly recent and started taking shape probably following introduction of experimental procedures in animals and isolation of active constituents from plants. Modern pharmacology uses information from physics, chemistry and biological sciences to synthesize new drugs and to determine what drugs do and how they do.

a. **Francois Magendie (1783-1855):** Francois Magendie was a French physiologist-pharmacologist, who in association with some of his pupils established the foundation for modern pharmacology. He is considered a pioneer of experimental physiology and pharmacology as he developed several experiments to elucidate physiological processes and action of drugs in the body. He studied effects of intravenous injections of ipecac, morphine, hydrocyanic acid, strychnine, iodine, potassium, veratridine, quinine and many other agents and published his work. He is known for describing the foramen of Magendie. Francois Magendie is also notoriously known for the cruel side of his experiments and dissection on live animals, which provoked an antivivisectionist campaign.

b. **Claude Bernard (1813-1878):** Claude Bernard was a French physiologist and a student of Francois Magendie. He is considered as 'Father of Experimental Medicine' for the use of scientific methods and experiments in medicine. He is mainly known for his discoveries concerning the role of pancreas in digestion, glycogenic function of liver, and regulation of blood supply by vasomotor nerves. He also identified the site of action of curare (arrow poison) and used curare, strychnine, carbon monoxide, etc. in his studies. Similar to the work of Francois Magendie, he subjected cruelty on animals in his experiments and practiced vivisection, which was opposed by his family and many scientists with campaign against the practice of vivisection.

c. **Rudolf Buchheim (1820-1879):** Rudolf Buchheim was a German pharmacologist who established the first laboratory devoted exclusively to the study of pharmacology at the University of Dorpat in Estonia. Buchheim is remembered for his pioneer work in experimental pharmacology and in turning pharmacology from an empirical study of medicine into an exact science. He conducted extensive research in his laboratory to elucidate actions of drug within the body. He introduced the bioassay to pharmacology and created a methodology for determining the quantitative and medical aspects of chemical substances. He also edited a book, eliminating obsolete and ineffectual medicines and practices, while adding updated information including a chapter of his own called *Art der Wirkung* (The Pharmacological Action). Today at the University of Giessen is the Rudolf Buchheim Institute for Pharmacology.

d. **Oswald Schmiedeberg (1838-1921):** Oswald Schmiedeberg was a German pharmacologist who established pharmacology as an independent scientific discipline based upon experimental methodology. He worked under Rudolf Buchheim at Dorpat and later on in 1872 became a professor of pharmacology in the Pharmacology Institute at the University of Strasbourg, France (then Germany) where he taught pharmacology for the next 46 years and trained about 150 pharmacologists. His work primarily dealt with finding the correlation between the chemical structure of substances and their effectiveness as narcotics. He introduced the technique of perfusing isolated organs and studied the effects of drugs on them. He worked on some of the most important drugs and poisons of that era including muscarine, nicotine, digitalis glycosides and various toxic heavy metals. For first time he demonstrated that muscarine had a similar effect on the heart as electrical stimulation of the vagus nerve. Schmiedeberg published over 200 scientific books and articles and is often regarded as the 'Father of Modern Pharmacology'.

e. **John Newport Langley (1852-1925)**: John Newport Langley was a British physiologist-pharmacologist, who determined in 1901 that adrenomedullary extracts (which contained both epinephrine and norepinephrine) elicited responses in different tissues which were similar to those induced by sympathetic nerve stimulation. In the wake of these findings, he proposed in 1905 that a receptive substance (now called receptor) was the site of action of chemical mediators liberated by nerve stimulation. The genesis of the concept of chemical synaptic transmission has been attributed to Langley

f. **John Jacob Abel (1857-1938):** John Jacob Abel was an American biochemist and pharmacologist. In 1891 he founded and chaired the first department of pharmacology in the United States at the University of Michigan. He is known for the isolation of epinephrine (adrenaline) in 1898 and later of insulin in crystalline form. He founded the 'Journal of Biological Chemistry'

in 1905 and 'Journal of Pharmacology and Experimental Therapeutics' in 1909. He is regarded as 'Father of American Pharmacology'.

g. **Sir Col. Ram Nath Chopra (1882-1973):** Sir Ram Nath Chopra was a pioneer in the field of experimental pharmacology of indigenous drugs of India. He became the first Professor of Pharmacology in India (1921) at Calcutta School of Tropical Medicine, and Head of the Department of Pharmacology at the Calcutta Medical College. He laid the foundation of scientific investigation on Indian indigenous drugs and was the Founder Director of the Drug Research Laboratory (presently Regional Research Laboratory) at Jammu. He is acclaimed as the 'Father of Indian Pharmacology'.

h. **L. Meyer Jones (1913-2002):** L. Jones Meyer is regarded as the 'Father of Modern Veterinary Pharmacology'. He authored the first edition of 'Veterinary Pharmacology and Therapeutics' in 1954 and was instrumental in shifting emphasis in the veterinary curriculum from materia medica to the modern science of veterinary pharmacology.

SCOPE OF PHARMACOLOGY

Pharmacology has a wide scope. It is an ever-changing discipline because as new research and clinical experience broaden our knowledge, changes in treatment and drug therapy also become necessary. With advancement in technology, many new diseases and disorders are being detected every day, which require new treatment strategies. The rapid development of resistant strains of many pathogenic organisms requires a continual need to develop new and better drugs to treat existing infections. Furthermore, increasing awareness among the livestock owners and public has laid more emphasis on the production and health, which are difficult to achieve without drugs.

As a science, pharmacology covers ground from basic screening of drugs to their safe application in the treatment of diseases. Therefore, over the years, various specialised sub-disciplines of pharmacology have been recognised. This essentially requires integration and understanding of other disciplines like physiology, pathology, microbiology, immunology, chemistry, biochemistry and various clinical specialties.

Table 1.1: Some important developments in Pharmacology and related disciplines since 1800 AD.

Year	Scientist(s)	Development
1807	Friedrich W. Serturner	Isolated morphine from opium.
1842	Crawford W. Long	Introduced ether as a general anaesthetic.
1853	Alexander Wood	Developed hollow hypodermic needle.
1869	Oswald Schmiedeberg	Isolated muscarine from *Amanita muscaria*.
1890	William Dymock, C.J.H. Warden and David Hooper	Published 'Pharmacographia Indica', a comprehensive book on the Indian medicinal plants.
1901	Hans H. Meyer and Charles E. Overton	Proposed separately the theory of oil/water distribution to explain anaesthetic justify action.
1908	Paul Gelmo	Synthesized sulphanilamide.
1914	Henry Dale	Identified the muscarinic and cholinergic actions of ACh. Awarded Nobel Prize (Jointly with Otto Loewi) in Physiology or Medicine in 1936 for this discovery.
1921	Otto Loewi	Showed that acetylcholine is a mediator of neuro-transmission. Awarded Nobel Prize in Physiology or Medicine (Jointly with Henry Dale) in 1936 for this discovery.
1923	Frederick G. Banting and Charles H. Best	Extracted insulin from the pancreas and demonstrated its hypoglycaemic effects. Frederick G. Banting was awarded Nobel Prize in Physiology or Medicine (Jointly with John J. R. Macleod) in 1923 for this discovery.
1928	Alexander Fleming	Discovered penicillin and found its curative effect in various infectious diseases. Awarded Nobel Prize in Physiology or Medicine (Jointly with Ernst B. Chain and Howard W. Florey) in 1945 for this discovery.
1932	Gerhard Domagk.	Introduced prontosil as the first clinical anti-streptococcal agent. Awarded Nobel Prize in Physiology or Medicine in 1939 for this discovery.
1939	Paul H. Muller	Discovered the high efficiency of DDT as a contact poison against several arthropods. Awarded Nobel Prize in Physiology or Medicine in 1948 for this discovery.
1944	Albert Schatz, Elizabeth Bugie, and Selman A. Waksman	Discovered streptomycin, the first effective antibiotic against tuberculosis. Selman A. Waksman was awarded Nobel Prize in Physiology or Medicine in 1952 for this discovery.
1948	Raymond P. Ahlquist	Postulated alpha- and beta-adrenergic receptors and established for first time that a single sympathetic mediator could produce both excitatory and inhibitory responses.
1950	-	The 'Central Drug Research Institute' was established at Lucknow, India.
1962	Earl W. Sutherland and Ted W. Rall	Demonstrated that adenosine 3',5'- cyclic adenosine monophosphoric acid (cAMP) functions as 'second messenger' in several hormonal actions.
1970	Bernard Katz , Ulf V. Euler and Julius Axelrod	Awarded Nobel prize in Physiology or Medicine for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanisms for their storage, release and inactivation.

Contd...

Year	Scientist(s)	Development
1971	J. R. Vane, J. B. Smith and A. L. Willis	Proposed separately that aspirin produces its action by inhibiting production of prostaglandins.
1982	Bengt I. Samuelsson, Sune K. Bergström and John R. Vane	Awarded Nobel Prize in Physiology or Medicine for their discoveries concerning prostaglandins and related substances.
1988	James W. Black , Gertrude B. Elion and George H. Hitchings	Awarded Nobel Prize in Physiology or Medicine for their discoveries of important principles for drug treatment.
1994	Alfred G. Gilman and Martin Rodbell	Awarded Nobel Prize in Physiology or Medicine for their discovery of the G Proteins and their role in signal transduction in cells.
1998	Robert F. Furchgott, Louis J. Ignarro and Ferid Murad	Awarded Nobel Prize in Physiology or Medicine for their discovery concerning nitric oxide (NO) as a signalling molecule in the cardiovascular system.
2005	Barry J. Marshall, and J Robin Warren	Awarded Nobel Prize in Physiology or Medicine for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease.

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CHAPTER 2 SOURCE, NATURE, NOMENCLATURE & CLASSIFICATION OF DRUGS

- Source of drugs
  - Natural source
  - Synthetic source
  - Semi-synthetic source
  - Biosynthetic source
- Nature of drugs
  - Alkaloids
  - Glycosides
  - Oils
  - Waxes
  - Resins
  - Tannins
  - Gums
  - Organic acids
  - Proteins
- Nomenclature of drugs
  - Chemical name
  - Non-proprietary name
  - Proprietary name
  - Other names
- Classification of drugs
  - Chemical nature
  - Source
  - Target organ/Site of action
  - Mode of action
  - Therapeutic use
  - Physiological system
  - Physical effect

A large number of drugs are used in clinics for the cure of various ailments. These drugs are diverse in chemical nature and are obtained from a variety of sources. In earlier days, simple chemical constituents and different parts of plants were employed as medicinal agents, but most drugs presently used in therapeutics are synthetic in nature.

SOURCES OF DRUGS

Drugs are obtained from many sources. These include natural, synthetic, semi-synthetic and biosynthetic.

1. Natural Source

From the ancient times, natural sources provide many important drugs. Some of these drugs are still used because they are efficacious, economical and easily available. The natural drugs come mainly from plants, animals, microorganisms and minerals.

a. **Plants:** Plants are the oldest source of drugs. The traditional Indian systems of medicine Ayurveda, Sidha and Unani are based primarily on the use of plants as well as some other natural substances. In modern pharmacology, plants continue to provide a number of well-established and important drugs (Table 2.1) in spite of significant progress in the area of development of new drugs from synthetic sources. Drugs provided by the plants are mostly having complex and highly varied structures which are unlikely to be synthesized in laboratories. Such plant drugs also act as source of chemical modifications needed for the production of semi-synthetic drugs. In addition to pure drugs, the crude extracts of some plants are used in therapeutics today. Discovery of several new life-saving anti-neoplastic drugs (e.g. etoposide, taxol and vinblastine) in recent past has renewed the interest of pharmaceutical industries in plants.

The biological activity of a plant is due to the presei of a specific or in certain cases more than one chemic constituents in it. These active constituents are genera isolated from the plant in crude form along with man inert or less active constituents and then purified various techniques to be used as a drug. The activ constituents are often more concentrated in a particu part of the plant, e.g. bark, fruit, flower, leaf, seed an root. In some cases, the entire plant may be used as source of the drug.

b. **Animals :** Animal source provides relatively few b important drugs, some of which are life-saving Synthesis of these animal derived drugs in laboratori is very cumbersome and expensive. Drugs from anima source, often called pharmazooticals, include antiser vaccines, hormones, enzymes, vitamins, coagulants anc anticoagulants, blood and blood components, etc. (Tab 2.2). Although genetically engineered drugs are rapidl replacing some of these pharmazooticals, but still fo many drugs there is no other source but the animals.

c. **Microorganisms :** Microbiology is a major participan in the global pharmaceutical and neutraceutical industry Microbial products comprising of actinomycetes, fungi and bacteria continued to play a highly significant rol in the drug discovery and development process. Drugs obtained from microbial sources include antimicrobials anti-neoplastics, vaccines, probiotics and vitamins. Antibiotics are valuable in the treatment agains infectious diseases and tumours, while vaccines provide immunity against them. Probiotics are thought to b healthy for the host organism by improving its intestinal microbial balance. Table 2.2 summarises some of th commonly used microbiological drugs.

Table 2.1: List of selected drugs derived from plant source.

Drug	Plant source (Botanical name)	Part of plant	Action/Clinical use
Arecoline	Areca palm (*Areca catechu)*	Nut (Seed)	Anthelmintic
Atropine	Deadly nightshade (*Atropa belladonna)*	Leaf/Seed	Anticholinergic
Camphor	Camphor tree *(Cinnamomum camphora)*	Wood	Rubefacient
Codeine	Opium poppy *(Papaver somniferum)*	Seed pod	Analgesic, antitussive
Colchicine	Colchicum (*Colchicum autumnale*)	Corn	Anti-gout.
Digitoxin/Digoxin	Foxglove plant (*Digitalis purpurea)*	Leaf	Cardiac stimulant
Emetine	Ipecacuanha *(Cephaelis ipecacuanha)*	Root	Emetic
Ephedrine/ Pseudoephedrine	Ephedra *(Ephedra vulgaris)*	Whole plant/Aerial parts	Sympathomimetic
Etoposide/Teniposide	Mayapple (*Podophyllum peltatum*)	Rhizome/Root	Anti-neoplastic agent
Hyoscine/Hyoscyamine	Henbane *(Hyoscyamus niger)*	Leaf/Seed	Anticholinergic
Morphine	Opium poppy *(Papaver somniferum)*	Seed pod	Analgesic
Physostigmine	Calabar bean *(Physostigma venenosum)*	Seed	Cholinesterase inhibitor
Pilocarpine	Indian hemp *(Pilocarpus jaborandi)*	Leaf	Anti-glaucoma
Pyrethrin	Pyrethrum *(Chrysanthemum spp.)*	Flower	Insecticide
Quinine	Cinchona *(Cinchona ledgeriana)*	Bark	Antimalarial
Rotenone	Derris *(Derris trifoliate)*	Root	Insecticide, piscicide
Scillarin A	Squill (*Urginea maritima*)	Bulb	Cardiac stimulant
Strychnine	Nux vomica (*Strychnos nux vomica*)	Seed	Rodenticide, Rumenotoric
Taxol	Pacific Yew (*Taxus brevifolia*)	Bark	Anti-neoplastic agent
Tubocurarine	Curare *(Chondrodendron tomentosum)*	Leaf/Root	Skeletal muscle relaxant
Vinblastine/Vincristine	Periwinkle *(Catharanthus roseus)*	Sap	Anti-neoplastic
Yohimbine	Yohimbe (*Pausinystalia yohimbe)*	Bark	Aphrodisiac, Amitraz poisoning

Table 2.2: List of selected drugs from animal and microorganism sources.

Type of drug	Examples
Animal source	
1. Antisera	Antitetanus serum, canine distemper serum and Haemorrhagic septicaemia serum.
2. Vaccines	Antirabies vaccine and polio vaccine.
3. Enzymes	Trypsin, chymotrypsin, pancreatin, pepsin and fibrinolysin.
4. Hormones	Posterior pituitary hormones, insulin, equine chorionic gonadotropin (eCG), thyroxine and calcitonin salmon.
5. Vitamins	Cod-liver oil and halibut liver oil.
6. Coagulants and anticoagulants	Absorbable gelatine sponge, aprotinin, heparin, protamine, eptifibatide and hirudin.
7. Blood and blood components	Whole blood, plasma, packed red cells and platelets.
8. Animal extractives and organs	Liver extract, bile and bile acids.
9. Anti-neoplastic and antiviral drugs	Cytosine arabinoside (Ara-C) and adenine arabinoside (Ara-A).
10. Miscellaneous drugs	Captopril, ziconotide and exenatide.
Microorganism source	
1. Antimicrobials	Bacitracin, penicillins, cephalosporins, streptomycin and amphotericin B.
2. Anti-neoplastics	Actinomycin D, daunorubicin, doxorubicin, bleomycin and mithramycin.
3. Vaccines	Anti-rabies vaccine, canine distemper vaccine and anthrax spore vaccine.
4. Anti-diabetic drugs	Acarbose, voglibose and valielamine.
5. Probiotics	*Lactobacillus* spp., *Bifidobacterium bifidus* and *Saccharomyces boulardii* (yeast).
6. Vitamins	B-Complex vitamins (from yeast).

d **Minerals :** Several minerals as simple element or their salts are important source of drugs since ancient times. Mineral containing substances are widely used in therapeutics and constitute economical source of drugs. They are also frequently used as dietary supplements to correct mineral deficiencies caused by various diseases, pregnancy, poor nutrition and certain medications. Minerals of medicinal value include iron, calcium, magnesium, sodium, potassium, sulphur and zinc. Table 2.3 summarises selected mineral drugs and their clinical use.

Table 2.3 : List of selected drugs from mineral source

Drug	Clinical use
Bismuth subnitrate	Antiseptic
Calcium carbonate	Antacid
Copper sulphate	Emetic
Ferric chloride	Styptic
Ferrous sulphate	Haematinic
Lead acetate	Antiseptic
Lithium carbonate	Antipsychotic
Magnesium sulphate	Purgative
Potassium bromide	Sedative
Potassium iodide	Expectorant
Silver nitrate	Caustic
Sodium bicarbonate	Antacid
Sulphur	Acaricide
Zinc sulphate	Astringent

2. Synthetic Source

Synthetic drugs are prepared by chemists in the laboratory from chemical substances. Most of the drugs produced by the pharmaceutical industry and used presently in therapeutics are synthetic drugs. With the advancement of synthetic pharmaceutical chemistry, many active constituents of plants have also been synthesised at less cost and in greater quantity than obtained from natural sources. Synthetic drugs are chemically pure and are often more effective and less toxic than the naturally obtained substances. They are easier to prepare in standardized units and can be manufactured in bulk to maintain a regular supply line. Examples of some widely used synthetic drugs are paracetamol, aspirin, diazepam, ofloxacin, sulphonamides, lidocaine and diphenhydramine.

3. Semi-synthetic Source

Semi-synthetic drugs are synthesized in laboratory utilizing a naturally occurring complex chemical as a starting material, thus obviating part of a total synthesis. Thus, these drugs are neither pure natural nor pure synthetic substances. The process of synthesizing a new drug by semi-synthetic method is extensively used in pharmaceutical industry nowadays because artificial synthesis of some drugs in laboratory is very difficult due to their complex molecule and/or expensive and uneconomical production. For example, plant products like steroid sapogenins are used as important raw material for the synthesis of steroidal hormones and related drugs. Some opioid analgesics like oxymorphone and hydromorphone have been produced by chemical modifications of natural morphine, while buprenorphine is semi-synthetically derived from thebaine. Several antibiotics such as ampicillin, amoxicillin and cephalosporins are also prepared from semi-synthetic source.

4. Biosynthetic Source

Biosynthetic source involves recently introduced techniques of genetic engineering or recombinant DNA technology. This innovative technology is now being used for large scale production of some complex polypeptide drugs, hormones, scarce enzymes and other proteins which are difficult to obtain in pure form from natural animal sources and are also difficult as well as very expensive to synthesise in the laboratory. Biosynthetic human insulin has been prepared by introducing coded DNA into *Escherichia coli*. Recombinant human erythropoietin (rhEPO), recombinant bovine somatotropin (bST) and recombinant feline rabies virus vaccine prepared from biosynthetic source are now available commercially for therapeutic use.

NATURE OF DRUGS

Various sources yield a large number of active constituents, which are used as drugs. These active constituents can be grouped according to their physical and chemical nature into numerous types. These include alkaloids, glycosides, oils, resins, tannins, organic acids and proteins.

1. Alkaloids

Alkaloids are group of complex organic compounds distributed widely in nature. These are obtained from plants (mainly), animals and fungi and are part of natural products. Many alkaloids have been prepared semi-synthetically and some even synthetically. Alkaloids possess significant physiological and pharmacological actions in man and animals and many are potent poisons. They possess following properties:

a. They are complex nitrogen containing organic compounds, generally having one or more heterocyclic rings.
b. They are mostly solid colourless crystalline compounds containing carbon, hydrogen, oxygen and nitrogen. A few alkaloids are colourless liquids, which generally do not possess oxygen.
c. They are alkaline in reaction (therefore named alkaloids).
d. They are readily soluble in alcohol, but are sparingly soluble in water. Their salts are soluble in water, which are used in therapeutics in preference to pure alkaloids.
e. Both alkaloids and their salts are precipitated by tannic acid and oxidized by potassium permanganate.
f. They are mostly bitter in taste and are often poisonous. These are, therefore, used in small doses in therapeutics.
g. Their name usually ends with suffix –ine.

Examples:

Plant source : Solid alkaloids – Atropine, morphine, quinine and strychnine.
Liquid alkaloids – Arecoline, nicotine and lobeline.
Animal source : Muscopyridine, epinephrine and serotonin.
Fungal source : Muscarine, ergotamine and ergometrine
Semi-synthetic source : Apomorphine, etorphine and homatropine.

2. Glycosides

Glycosides are complex organic compounds in which a sugar is bound to a non-sugar moiety. Glycosides mainly exist in plants, but a few are also found in animals. Many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis. Glycosides play important role in therapeutics and some are potent poisons.

a. They are complex organic compounds having sugar(s) attached to a non-sugar part mainly by an ether linkage. The sugar group is known as glycone and the non-sugar group as aglycone or genin.
b. They mainly contain carbon, hydrogen and oxygen. In addition, some glycosides have nitrogen and a few have sulphur.
c. They are neutral in reaction.
d. They are mostly soluble in alcohol, less soluble in water and insoluble in ether. They do not combine with acids to form salts.
e. They upon acid hydrolysis in body liberate aglycone and one or more sugars. If the liberated sugar moiety is glucose, they are called glucoside and when it is galactose they are categorized as galactoside.
f. Some glycosides are pharmacologically very active, while others are practically inert. The pharmacological activity of glycosides primarily resides in the non-sugar moiety; the sugar moiety is not essential for the pharmacological activity but it governs the pharmacokinetic properties of the glycoside.
g. Their name usually ends with suffix –in.

Examples: Digitoxin, digoxin, ouabain and scillarin.

Saponins

Saponins are generally the plant glycosides (aglycone linked to sugar) having distinctive property of frothing. They produce foam when shaken with water and produce stable emulsion on shaking with oils and fats. The aglycone moiety of the saponins, often called sapogenins, is either a triterpene or steroid. Some saponins are toxic and are known as sapotoxins. Saponins are now classed as a subgroup of glycosides.

Examples: Digitonin, senegin and glycyrrhizin.

3. Oils

Oils are diverse chemical substances obtained from plant, animal or mineral sources. They are mostly liquids and are insoluble in water. Broadly they are of three types: volatile, fixed and mineral oils.

a. Volatile Oils

Volatile oils (also called essential, ethereal, aromatic or flavouring oils) are a diverse group of aromatic chemical compounds produced mainly by plants. They are usually responsible for the odour of a plant and are obtained by process of steam distillation. Volatile oils are used for various medicinal purposes.

a. They are complex mixtures of diverse chemical compounds such as terpenes, alcohols, aldehydes, ketones, esters and phenols.
b. They are secreted by specialized cells in some plant parts, mainly the flowers, leaves and fruits and give a characteristic aroma (smell) to plants.
c. They are mostly colourless liquids. Some of these oils are crystalline or amorphous solid at room temperature and sublime on heating.
d. They are less soluble in water, but highly soluble in alcohol, ether and other organic solvents. Alcoholic solutions of volatile oils, called essences, are frequently used in perfumery.
e. They evaporate on exposure to air at ordinary temperature and leave no greasy stain.
f. They are obtained mainly by process of distillation without being decomposed.
g. They do not form soap with alkalis.
h. On prolonged stay, they do not become rancid. However on exposure to light, they tend to oxidise and turn rancid.
i. They have no caloric or food value.

Examples:

Liquid volatile oils : Eucalyptus oil, clove oil and peppermint oil.
Solid volatile oils : Camphor, menthol and thymol.

Empyreumatic oils

Empyreumatic oils are volatile oils which do not exist in the living plants, but are formed subsequently by destructive distillation.

Examples: Creosote and oil of tar.

b. Fixed Oils

Fixed oils are esters of glycerol with long chain fatty acid(s) called glycerides. They are widely distributed and occur both in plants (mainly) and animals.

a. They are glycerides of stearic, oleic and palmitic acids.
b. They are chiefly obtained from seeds, occurring within the cells as droplets or crystals.
c. They are liquid at ordinary temperature.
d. They are non-volatile, and as such cannot be distilled without decomposition. Therefore, they are obtained by process of expression.
e. They are insoluble (immiscible) in water, sparingly soluble in alcohol and freely soluble in ether.
f. They leave a permanent greasy stain.
g. They form soap with alkalis.
h. On heating or prolonged stay, they become rancid.
i. They possess caloric or food value.

Examples:

Plant fixed oils : Olive oil, castor oil, arachis oil and linseed oil.
Animal fixed oils : Cod liver oil and shark liver oil.

Fats

Fats are fixed oils, which remain solid or semisolid at ordinary temperature. They contain more of palmitin and stearin, and are obtained mainly from animal source.

Examples: Lard, lanolin and butter.

c. Mineral Oils

Mineral oils (Petrochemical oils) are mostly petroleum products extracted by fractional distillation. They are obtained from underground petroleum-bearing reservoirs and, therefore, do not belong to the class of organic oils.

a. They are mixture of hydrocarbons of the methane and related aliphatic series.

b. They are obtained as a by-product in the distillation of petroleum to produce gasoline and other petroleum based products from crude oil.
c. They are produced in various consistencies (liquid or semisolid) and have diverse uses. Liquid mineral oils are mostly transparent and colourless.
d. They do not form soap with alkalis.

Examples: Hard paraffin, soft paraffin and liquid paraffin.

4. Waxes

Wax is a type of lipid secreted by plants and animals, but now mostly prepared synthetically or semi-synthetically by purification from natural petroleum. They are generally distinguished from fats and oils by having a melting point above approximately 45°C.

a. They are esters of higher alcohols (e.g. cetyl alcohol) with higher fatty acids (called wax esters).
b. They are firm in consistency and have high melting point above approximately 45 °C.
c. They are insoluble in water, but are soluble in many organic solvents.
d. They have a relatively low viscosity when melted.
e. They can be saponified by alcoholic alkali.

Examples: Bees wax and paraffin wax.

5. Resins

Resins are ill defined solid substances found in plants, produced by oxidation and polymerization of volatile oils. The term is also used for synthetic substances of similar properties.

a. They are viscous secretions of many plants, particularly coniferous trees, formed in specialized passages or tubes called resin ducts and are often collected by giving incision on a particular area of the plant.
b. They are mixture of various substances of complex chemical nature like acids, alcohols and esters.
c. They exist as amorphous and brittle solids.
d. They are insoluble in water, but are soluble in organic solvents such as alcohol, chloroform and ether.
e. They are soluble in alkalis forming non-detergent resin soaps.

Examples: Jalap and podophyllum.

Oleoresins

Oleoresins are semisolid mixtures of resins with volatile oils. They are natural secretions of plants and may vary considerably in their composition.

Examples: Colophony and crude turpentine.

Gum-resins

Gum-resins are mixtures of resins with gums.

Example: Asafoetida.

Balsams

Balsams are oleoresins containing benzoic acid or cinnamic acid, or both.

Examples: Benzoin, balsam of Peru and balsam of Tolu.

6. Tannins

Tannins are complex non-nitrogenous phenolic compounds, which have an astringent action on the mucous membranes. They are widely distributed in many species of plants, where they play a role in protection from predation and perhaps also in growth regulation.

a. They are non-nitrogenous polyphenolic compounds found mainly in plants.
b. They occur mainly in leaves, roots and bark of various plants, often in solution in the cell sap in distinct vacuoles.
c. They cause precipitation of proteins and thus exert an astringent action on the mucous membranes.
d. They possess irritant and bitter taste.
e. They are soluble in water.
f. They react with iron compounds to yield purple, violet or black colour precipitates. They also precipitate metallic salts and alkaloids.

Tannins are broadly of two sub-types: pyrogallol tannins and pyrocatechol tannins.

Pyrogallol tannins

Pyrogallol tannins are derived from the trihydric phenol pyrogallol. They are generally glycosides of glucose and occur mainly in oak galls, clove, chestnut, etc.

Examples: Tannic acid (tannin obtained from oak gall).

Pyrocatechol tannins

Pyrocatechol tannins are derived from the dihydric catechol. They do not contain sugar and occur mainly in catechu, cinnamon and cinchona.

7. Gums

Gums are secretory products of plants, which are used as emulsifying agents in oil and suspending agents for insoluble substances.

a. They are polysaccharides, which on hydrolysis yield simple sugars.
b. They are amorphous, colloidal exudates from plants found mainly in the woody elements or seed coatings.
c. They are insoluble in water.
d. Upon addition of water, some gums swell up and form viscid adhesive fluid called mucilage; others remain unchanged.

Examples: Agar, psyllium, gum tragacanth and gum acacia.

8. Organic Acids

Organic acids are organic compounds with acidic properties found in plants either in combination with bases like potassium, calcium or magnesium, or in a free state as acids.

a. They are weak acids and do not dissociate completely in water.
b. Low molecular weight organic acids (e.g. formic and lactic acids) are miscible in water, but high molecular weight organic acids (e.g. benzoic acid) are insoluble in molecular (neutral) form. Most organic acids are very soluble in organic solvents.
c. They are much less reactive with metals than are strong mineral acids like HCl

Examples: Citric acid, tartaric acid, benzoic acid and salicylic acid.

9. Proteins

Proteins are macromolecules consisting of long sequences of a-amino acids in peptide linkage. They are present in various forms in all living cells of animals and plants. Proteins are not commonly used in therapeutics due to their biodegradation in the gastrointestinal tract and due to producing undesirable pharmacological effects.

Examples: Albumin and globulins.

NOMENCLATURE OF DRUGS

Nomenclature implies assigning proper name(s) to a particular compound so that its study, marketing or use may become systematic. Most drugs available in market have at least three different names: chemical name, non-proprietary name and proprietary name.

1. Chemical Name

Chemical name is a name given to a drug in accordance with rules of chemical nomenclature established by naming conventions like International Union of Pure and Applied Chemistry (IUPAC) or Chemical Abstracts Service (CAS). The chemical name is useful to the chemists, scientists or technical personnel as it provides the precise arrangement of atoms and atomic groups in the molecule and the chemical constitution of the drug. However, the chemical name of drugs is generally too complex to be widely used or remember for prescribing or marketing the drugs except for some simple compounds like diethyl ether or sodium chloride. Moreover, there are several naming conventions or systems used by chemists so that one drug may have several widely differing but correct chemical names.

2. Non-proprietary Name

Non-proprietary name is a short name given to a drug that is not subject to proprietary (trademark) rights. Non-proprietary name is easy to speak and spell, and is often designated to identify a particular drug entity. Non-proprietary name replaces the long, unwieldy chemical name for a pharmaceutical substance and is frequently used in textbooks and other discussions in pharmacology. The non-proprietary name is also referred to as the generic name, but etymologically this is incorrect as the term generic is more appropriate for chemical or pharmacological group (or genus) of drugs such as sulphonamides, penicillins and benzodiazepines. There are two classes of non-proprietary names: approved name and official name.

Approved name

Approved name (Adopted name) is the name given to a drug by drug nomenclature bodies like United States Adopted Names (USAN) Council soon after its introduction. The American system of drug nomenclature is called the United States Adopted Name (USAN), whereas that of the United Kingdom is called British Approved Name (BAN). An International Non-proprietary Name (INN), also known as recommended International Non-proprietary Name (rINN), is the official non-proprietary name given to a pharmaceutical substance, as recommended by the World Health Organization (WHO). The approved name of the drug may or may not be included in the pharmacopoeia.

Official name

Official name is the name approved by the National Pharmacopoeia Commission and is included in the official book Pharmacopoeia. The official name may not be approved immediately after introduction of a new drug, but rather when the drug is considered to have established itself. At present, there are several pharmacopoeias (e.g. U.S.P., B.P. and I.P.) and each national commission is free to formulate its own official name. The official name may be identical with the approved name.

The non-proprietary names are usually derived from the full chemical names of the compounds by a process of contraction keeping in mind the pharmacological and/or chemical relationships of drugs. The non-proprietary names do not confuse with other existing drugs and generally indicate relationship with the existing similar drug (e.g. cloxacillin, ampicillin and amoxicillin) and possibly class of drugs (e.g. sulphonamides, penicillins and benzodiazepines). Earlier the non-proprietary names used to vary from one country to other, depending on the agency and country coining it. For example, an opioid analgesic drug was having non-proprietary name of pethidine in the United Kingdom, meperidine in the United States of America, and dolantin in the Germany. Similarly, barbiturates had a suffix -one in the U.K. and -al in the U.S.A., e.g. pentobarbitone (U.K.) was the same drug as pentobarbital (U.S.A.). However, according to a recent agreement, all member countries of WHO have decided to use the uniform recommended International Non-proprietary Name (rINN, INN) for drugs used in their countries. The rINN shall be applicable to all newer as well as to older drugs, with a few exceptions. The BAN of several older drugs (e.g. noradrenaline and lignocaine) has now been modified to be commensurate with rINN. Keeping in mind the changed scenario, the recommended International Non-proprietary Names are also used for drug names in the present book with former British Approved Names and United States Adopted Names given as synonyms.

Name stems

Stem is a term used to define the pharmacologically related group to which the drug belongs. The stems are selected by WHO experts and are mostly used as suffix, but in some cases as prefix. Drugs from the same therapeutic or chemical class are usually given INNs with the same stem so that all substances belonging to the same group can be easily recognized. Names those are likely to convey an anatomical, physiological, pathological or therapeutic suggestion are generally avoided. Some common stems used for drugs of the same class are: -coxib for COX-2 inhibitors (e.g. celecoxib and rofecoxib), -peril for ACE-inhibitors (e.g. captopril and benazepril), -azepam for anxiolytics (e.g. diazepam and lorazepam), -caine for local anaesthetics (e.g. procaine and lidocaine), and –olol for beta adrenoceptor blockers (e.g. propranolol and esmolol).

3. Proprietary Name

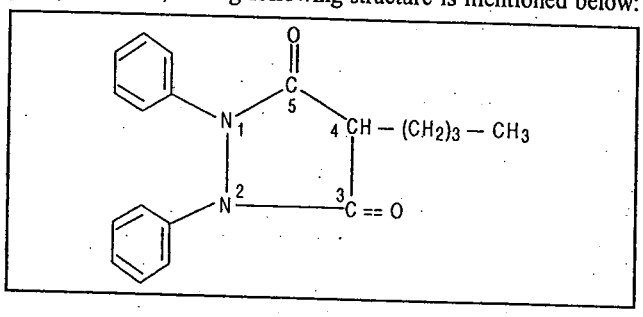
Proprietary (Brand, trade or registered) name(s) is a name given to a drug by the pharmaceutical firm which sells the drug, although it may not have manufactured the drug itself. Thus, a single drug may be sold under many proprietary names by different firms. Even a single pharmaceutical firm may give different proprietary names to a single drug having different formulations. The pharmaceutical company, which sells the non-proprietary drug, selects the proprietary name according to its marketing convenience and gets it registered and the trade name then becomes the sole property of the pharmaceutical company. Proprietary names are written with a capital initial letter and are often further distinguished by a superscript R in a circle(®). The

proprietary names are not suitable for scientific studies and are not included in textbooks. The clinicians usually prescribe drugs by their proprietary names.

4. Other Names

In addition to its formal chemical and non-proprietary names, a drug may have a code designated name. Code name is provided by the pharmaceutical manufacturer before the drug is placed on the market. Code designated name helps the manufacturer to maintain secrecy during drug development and pre-marketing investigational stages. In addition, Chemical Abstract Service Registry Number (CAS RN), a unique reference number, is provided to all chemical moieties including each salt, hydrate, isomer and racemic form of the drug.

Example: The complete nomenclature for a single drug, phenylbutazone, having following structure is mentioned below:



Chemical structure

a.	Chemical name	N-Butyl-3, 5-dioxo-1, 2,-diphenyl pyrazolidine
b.	Non-proprietary name	Phenylbutazone
c.	Proprietary names (Veterinary preparations)	Butazolidin (Schering) Butalon (Apex) Butaron (Rhone Merieux) Equipalazone (Hoechst Roussel Vet.) Companazone (Arnolds) Phenycare (Animal Care)

CLASSIFICATION OF DRUGS

Classification of drugs is essential because it allows several thousands of drug substances to be reduced to a manageable number of groups about which generalisations can be made. However, there is no uniform or homogenous system of classifying drugs which suits all purposes. Drugs are generally classified according to the convenience of the person discussing them viz. clinician, pharmacologist, chemist, pharmacist, etc. For example, classification of drugs according to their chemical nature or structure may be useful for a medicinal chemist, but it does not provide a meaningful classification scheme to a pharmacologist interested in drug effect as it is well known that many drugs with similar chemical structures produce much different effects and, on the other hand, many drugs which differ in chemical structure produce similar biological effects. Nevertheless, classification systems which take into account both the chemical and biological properties of the agent and also the therapeutic use are likely to be useful for most professionals including pharmacologists and clinicians. This section describes several common schemes for classifying drugs; each drug can be classified into one or more drug classes.

1. Chemical Nature

a. **Inorganic drugs**
   - (*i*) Metals and their salts
     e.g. ferrous sulphate, magnesium sulphate and zinc sulphate.
   - (*ii*) Non-metals
     e.g. sulphur, oxygen and hydrogen peroxide.

b. **Organic drugs**
   - (*i*) Alkaloids
     e.g. atropine, morphine, strychnine and cocaine.
   - (*ii*) Glycosides
     e.g. digitoxin, digoxin and ouabain.
   - (*iii*) Peptides/Proteins
     e.g. insulin, oxytocin and vasopressin.
   - (*iv*) Esters
     e.g. suxamethonium, procaine and acetylcholine.
   - (*v*) Amides
     e.g. lidocaine and procainamide.
   - (*vi*) Alcohols
     e.g. ethyl alcohol.
   - (*vii*) Glycerides
     e.g. castor oil, linseed oil and olive oil.

2. Source

a. **Natural source**
   - (*i*) Plant source
     e.g. morphine, atropine, digitoxin and physostigmine.
   - (*ii*) Animal source
     e.g. heparin, lanolin and equine chorionic gonadotropin.
   - (*iii*) Microbiological source
     e.g. penicillin-G, streptomycin and griseofulvin.
   - (*iv*) Minerals and their salts
     e.g. sodium chloride, ferrous sulphate and copper sulphate.

b. **Synthetic source**
   e.g. halothane, procaine and sulphanilamide.

c. **Biosynthetic source**
   e.g. recombinant human erythropoietin and recombinant bovine somatotropin.

3. Target Organ/Site of Action

It is difficult to classify a drug on the basis of its target organ or system as it may affect other systems also. The action on the primary site has been taken as a basis of classification in the present scheme.

a. Drugs acting on central nervous system
   e.g. phenobarbital, diazepam and phenytoin.
b. Drugs acting on respiratory system
   e.g. bromhexine, clenbuterol and ipratropium.
c. Drugs acting on cardiovascular system
   - (*i*) Heart
     e.g. digitoxin, digoxin and milrinone.
   - (*ii*) Blood vessels
     e.g. hydralazine, nifedipine and nitroprusside.

d. Drugs acting on gastrointestinal tract
   - (*i*) Stomach
     e.g. omeprazole, pentagastrin and cimetidine.

(*ii*) Intestine
e.g. sulphasalazine, carboxymethylcellulose and phenolphthalein.

4. Mode of Action

a. Calcium channel blockers
e.g. verapamil, diltiazem and nifedipine.
b. Anticholinesterase agents
e.g. neostigmine and physostigmine.
c. Histamine receptor antagonists
e.g. diphenhydramine, pheniramine and cetirizine.
d. Inhibitors of bacterial wall synthesis
e.g. penicillins, cephalosporins and bacitracin.

5. Therapeutic Use

a. Antimicrobials
e.g. penicillins, cephalosporins, chloramphenicol and sulphonamides.
b. Antihypertensives
e.g. clonidine, hydralazine and enalapril.
c. Antidiarrhoeals
e.g. loperamide, kaolin and diphenoxylate.
d. Anticonvulsants
e.g. phenytoin, valproic acid and carbamazepine.
e. Antiemetics
e.g. domperidone, ondansetron, meclozine and metoclopramide.

6. Physiological System

a. Sympathomimetics
e.g. epinephrine, norepinephrine and isoprenaline.
b. Parasympathomimetics
e.g. carbachol, pilocarpine and neostigmine.
c. Neuromuscular blockers
e.g. suxamethonium, gallamine and d-tubocurarine.
d. Anticoagulants
e.g. heparin and warfarin.

7. Physical Effect

a. Emollients
e.g. lanolin, petroleum jelly and bees wax.
b. Counterirritants
e.g. methyl salicylate, menthol and cantharidin.
c. Caustics
e.g. silver nitrate and antimony trichloride.
d. Adsorbents
e.g. activated charcoal and magnesium trisilicate.
e. Protectives
e.g. starch, calamine and talc.
f. Surfactants
e.g. dimethicone and turpentine oil.

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CHAPTER 3

DOSAGE FORMS AND ROUTES OF DRUG ADMINISTRATION

Dosage forms
Types of dosage form
Oral dosage forms
Parenteral dosage forms
Inhalant dosage forms
Topical dosage forms
Special types of dosage form
Routes of drug administration
Factors affecting route of drug administration
Routes of drug administration
Oral route
Parenteral routes
Inhalation
Topical administration
Specialised drug delivery systems
Fixed dose combination preparations

DOSAGE FORMS

Dosage form (Drug preparation) is the physical form in which a drug or medication is compounded for administration by various routes to produce desirable pharmacological effect. Various dosage forms are recognised by the pharmacopoeias and other drug compendia and are widely used in therapeutics. Multiple dosage forms may exist for a single particular drug, since different therapeutic conditions can require different routes of administration. Additionally, a specific dosage form may be a requirement for certain kinds of drugs to provide chemical stability, target specific delivery or controlled effect. Suitable dosage forms are needed for one or more of the following purposes:

a. To deliver drug in a convenient, painless and acceptable manner.
b. To facilitate absorption of drug in a predictable and desirable manner.
c. To deliver active principle(s) in a stable form.
d. To deliver drug at the target site (e.g. enteric coated tablet).
e. To provide protection against moisture, light and atmospheric oxygen (e.g. coated tablet and capsule).
f. To provide protection against gastric acid (e.g. enteric coated tablet).
g. To mask bitter taste or nauseant odour of the drug (e.g. capsule and coated tablet).
h. To make good taste or flavour (e.g. syrup and aqua).
i. To mix two immiscible substances (e.g. emulsion).
j. To provide long shelf-life.
k. To provide uniformity with respect to drug contents.
l. To provide extended drug action through controlled release mechanisms (e.g. pellet).

Types of dosage form

The dosage forms can be classified in a number of ways depending on mode of administration, physical properties, mechanical processes used, types of vehicle used, etc. The most suitable classification of dosage forms commonly used in veterinary practice appears to be that based on mode of administration.

I. Oral dosage forms
  1. Liquids
     e.g. aqua, elixir, emulsion, extract, gel, linctus, mixture, solution, syrup and tincture.
  2. Solids and semi-solids
     e.g. block, bolus, capsule, granule, pill, powder and tablet.

II. Parenteral dosage forms
  1. Liquids
     e.g. injection and serum.
  2. Solids
     e.g. implant and pellet.

III. Inhalant dosage forms
  1. Gases
     e.g. aerosol, gas and inhalant.

IV. Topical dosage forms
  1. Liquids
     e.g. douche, drops, enema, liniment, lotion, paint and shampoo.
  2. Solids and semisolids
     e.g. cream, dusting powder, electuary, insufflation, jelly, ointment, paste, patch, plaster, poultice, sponge and suppository.
  3. Gases
     e.g. spray.

I. Oral Dosage Forms

1. Liquids

a. **Aqua:** Aqua (Aromatic water, medicated water) is a weak and simple solution of volatile substances in water. It is

mainly used as flavouring agent or vehicle e.g. peppermint water and camphor water.

b. **Elixir:** Elixir is an aromatic and sweetened hydroalcoholic solution generally containing dissolved drugs. It is often used as vehicle to mask disagreeable odour and taste of many drugs e.g. simple elixir and elixir cascara sagrada.

c. **Emulsion:** Emulsion is a suspension of two immiscible liquids (oil and water) in which one of the liquids is finely divided by trituration and uniformly distributed in the other by means of an emulsifying agent. It is designed mainly to disguise the taste or oiliness of medicinal oils and to improve their absorption e.g. castor oil emulsion.

d. **Extract:** Extract is a concentrated liquid, semi-solid or solid preparation of plant or animal drug extracted with a suitable solvent. Depending on the consistency, extracts are of following three subtypes:

(*i*) Liquid extracts e.g. liquid extract of belladonna.

(*ii*) Semi-solid or soft extracts e.g. glycyrrhiza extract.

(*iii*) Solid or dry extracts e.g. belladonna siccum extract.

e. **Gel:** Gel is an aqueous colloidal suspension of the hydrated form of insoluble drugs. It provides good protective and soothing coating over inflamed mucosa and may also be considered suitable adsorbent and antacid e.g. aluminium hydroxide gel.

f. **Linctus:** Linctus is a viscous and sweet preparation of drugs having demulcent action on the mucosae of throat. It contains a high proportion of syrup and, sometimes, glycerine. It is mainly used in the treatment of irritating cough.

g. **Mixture:** Mixture is a preparation in which drugs are simply dissolved or suspended in water for oral administration e.g. magnesium hydroxide mixture and carminative mixture.

h. **Solution:** Solution is a clear homogenous preparation of non-volatile drugs dissolved in water or any other suitable solvent. It is used for many purposes and by many routes.

(*i*) Oral solutions e.g. morphine hydrochloride solution and calciferol solution.

(*ii*) Parenteral solutions e.g. sodium chloride solution (0.9%).

(*iii*) Topical solutions e.g. iodine solution and hydrogen peroxide solution.

i. **Syrup:** Syrup is a liquid preparation of drugs in which the vehicle is concentrated aqueous solution of sucrose or any other sugar. The term has also been used to include any other liquid oral dosage form that is prepared in a sweet and viscid vehicle, including oral suspensions. Syrup may be medicated syrup (used as stock solution) or flavouring syrup (used as vehicle or flavouring agent) e.g. simple syrup and lemon syrup.

j. **Tincture:** Tincture is an alcoholic or hydroalcoholic solution containing active principles of plant drugs (mainly) or chemical substances. It is relatively weak compared with liquid extract. Tincture is very stable preparation that can be stored for long time without decomposition, degradation or loss of potency due to preservative action of alcohol in it e.g. tincture belladonna and tincture digitalis.

2. Solids and Semisolids

a. **Block:** Block is a solid preparation usually in the shape of square or rectangle containing nutrients or drugs meant for ingestion by licking e.g. mineral mixture block.

b. **Bolus:** Bolus (Ball) is a cylindrical mass of drugs which weighs about 60 g, measures about 2½ inches long and ½ inch in diameter and is slightly tapering e.g. stomachic bolus. Presently the term bolus is used mainly for a large compressed tablet, usually rectangular in shape. Bolus is used for horses and cattle to provide large amount of drugs required in a single dose.

c. **Capsule:** Capsule is an oral preparation consisting of a shell and a filling containing a solid, liquid or semi-solid drug. The capsule shell may be composed of a single sealed enclosure, or two halves which fit together and are sometimes sealed with a band. Capsule shell is made from gelatin, starch, cellulose or other suitable material and is of two types: hard shell (hard capsules) for solid drugs and soft shell (soft capsule) for solid, liquid or semisolid drugs.

(*i*) Hard capsule e.g. amoxicillin capsule.

(*ii*) Soft capsule e.g. vitamin E capsule.

d. **Granule:** Granule is a globular particle or grain in which drugs are mixed with sugar, flavouring agent and inert adjuvant. It is used mainly for administering drugs those possess unpleasant taste e.g. methylcellulose granule.

e. **Pill:** Pill is a spherical or ovoid usually sugar coated preparation intended to be swallowed without being chewed. Now pill is less used because it often becomes hard on drying and may not easily dissolve in the gastrointestinal tract.

f. **Powder:** Powder is a preparation containing drugs in dried and finely divided (pulverulent) form that may be intended for internal (powder for oral administration) or external use (dusting powder). Powder for internal use gives faster dispersal and absorption of drugs in the GI tract and is a convenient form to dispense bulky drugs having large doses. In small animals, powders are generally employed by adding to the drinking water or feed e.g. triple bromide powder and antidiarrhoeal powder.

g. **Tablet:** Tablet is a small disc shaped preparation usually made by moulding or compressing the granulated or powdered drugs. Tablet varies in shape and size, and may be scoured on both surfaces to facilitate fractionisation for providing smaller doses e.g. enrofloxacin tablet.

II. Parenteral Dosage Forms

1. Liquids

a. **Injection:** Injection (Sterilised injectable preparation) is a sterile solution or suspension of drugs for parenteral administration. Some drugs are unstable in liquid form; therefore they are supplied as powder for reconstitution with sterile solvent immediately before use for injection (Injection powder for solution/suspension). Injections

must be prepared and administered in a manner that maintains sterility e.g. gentamicin injection and penicillin-G injection.

b. **Serum:** Serum is preparation of blood serum containing antibodies for specific antigens. It is indicated promptly after suspected exposure to a toxicant e.g. antitetanic serum (ATS).

2. Solids

a. **Implant:** Implant is a solid material inserted or grafted surgically into intact body tissues. It may include medicated bodies, tissue grafts and artificial pacemakers. Medicated implant is generally prepared as a rod, cylinder, spherical pellet or compressed tablet and releases the drug at a constant rate for a predetermined period of time e.g. implants of naltrexone (for opioid dependent addicts in human medicine), diethylstilbestrol and anabolic steroids (as growth promotant).

b. **Pellet:** Pellet is a very small ovoid or spherical sterile solid mass of drugs, usually of steroid hormones, meant for implantation in subcutaneous tissues by incision. It serves as repository or depot dosage form providing the sustained release of drugs over extended period of time e.g. testosterone pellet.

III. Inhalant Dosage Forms

1. Gases

a. **Aerosol:** Aerosol is a suspension of fine, solid or liquid drug particles packaged in a container under pressure in the gaseous form. The drug is released upon activation of an appropriate valve system in the form of a mist or fog. Pressurised aerosol container may have a metering device that delivers the measured dose. Aerosol is intended for topical application to the skin as well as local application into the nose (nasal aerosol), mouth (lingual aerosol) or lungs (inhalation aerosol) e.g. terbutaline aerosol.

b. **Gas:** Gas is any elastic aeriform fluid in which the molecules are separated from one another and able to move freely. Gas is intended to be used directly by inhalation e.g. oxygen, carbon dioxide and nitrous oxide.

c. **Inhalant:** Inhalant or Inhalation is a preparation consisting of or containing volatile drugs which are carried by an air current into the nasal passage to relieve congestion and inflammation of the respiratory tract. Some substances are volatile at room temperature and may be inhaled directly, whereas others may be put in hot water and vapours are inhaled e.g. menthol inhalation.

V. Topical Dosage Forms

. Liquids

a. **Douche:** Douche (Irrigant) is a sterile solution of drugs used for rinsing the body cavities, mainly to wash out and treat infection of vagina. It is administered via a thin, soft, rubber or plastic tube known as catheter. The term irrigant is also used for a sterile solution intended to bathe or flush open wounds.

b. **Drops:** Drops is a liquid preparation in which a drug solution or suspension is instilled into body cavities such as ear, nose, mouth and eye in drop-wise fashion with a dropper. Drops must be sterile and prepared under the same conditions and by the same methods as parenteral preparations e.g. sulphacetamide eye drops and gentamicin eye/ear drops.

c. **Enema:** Enema is liquid preparation meant for administration into the rectum for purpose of providing the drug or clearing out the bowel. It is performed either with an enema can or an enema pump in horses and cattle, and with a human enema syringe in small animals e.g. soap water enema.

d. **Liniment:** Liniment is a liquid or semi-solid preparation of drugs in oil, alcohol or soap intended for external application on intact skin by inunction (rubbing). Generally it contains camphor and possesses rubefacient, analgesic or stimulating properties e.g. camphor liniment and turpentine liniment.

e. **Lotion:** Lotion is a liquid preparation of drugs, usually in water, meant for external application on skin without inunction. It is generally used for local soothing, cooling or protective purposes e.g. white lotion and calamine lotion.

f. **Paint:** Paint is a liquid preparation of drugs in viscid solvent such as glycerine and/or liquid paraffin for application to skin or mucous membranes, usually with a soft brush. Throat paint is more viscous due to a high content of glycerine, which being sticky adheres to the affected site and prolongs the action of drugs e.g. Mandl's throat paint.

g. **Shampoo:** Shampoo is a liquid soap or detergent used to clean the hair and scalp and is often used as a vehicle for dermatological agents. The medicated shampoo contains one or more drugs and is used mainly to treat various skin disorders such as irritation, itching, dandruff, seborrhea, minor bacterial and fungal infections and ectoparasites e.g. hydrocortisone shampoo and ketoconazole shampoo.

2. Solids and semisolids

a. **Cream:** Cream is a soft semi-solid emulsion preparation of drugs which usually contains more than 20% water and volatiles and/or less than 50% hydrocarbons, waxes, or polyols as the vehicle. It is intended for gentle rubbing over the skin or mucous membranes e.g. calamine cream and nystatin cream.

b. **Dusting powder:** Dusting powder is a preparation of drugs in fine powder form for application to skin or mucous membranes by process of dusting. It may be applied for the protective and lubricant properties on intact skin (medical powder) or the adsorbent and antibacterial properties on wounds and body cavities (surgical powder).

(*i*) Medical dusting powder e.g. talcum powder and iodoform powder.

(*ii*) Surgical dusting powder e.g. corn starch and antimicrobial dusting powder.

c. **Electuary:** Electuary is semisolid preparation of drugs (medicinal paste) mixed with sugar, treacle or honey

intended for application by smearing on the tongue, lips and palates. It provides demulcent action over the inflamed mucous membranes and ensures slow release of drugs for longer period. It is mainly used in large animals for treatment of respiratory diseases e.g. saline electuary.

d. **Insufflation:** Insufflation is a medicated dusting powder that is blown by an insufflator into body cavities such as nose, throat, vagina, ears, etc. to which it would be difficult to apply the powder directly.

e. **Jelly:** Jelly is a transparent or translucent, non-greasy semisolid preparation meant for external application to the skin or mucous membranes. It is generally prepared either from natural gums like tragacanth, pectin and sodium alginates, or from synthetic derivatives of natural substances like methylcellulose and sodium carboxymethylcellulose e.g. tragacanth jelly and sodium alginate jelly.

f. **Ointment:** Ointment is a semisolid preparation of drugs in oily, fatty or paraffin bases meant for external application to the skin or mucous membranes. It differs from cream in containing less than 20% water and volatiles and more than 50% hydrocarbons, waxes or polyols as the vehicle. It provides a means of applying drugs for prolonged local effect and is not used on discharging wounds because they prevent free drainage e.g. iodine ointment, sulphur ointment and Whitfield ointment.

g. **Paste:** Paste is a semisolid preparation of drugs which contains a large proportion (20-50%) of finely divided solids dispersed in a fatty vehicle. It is generally very thick and stiff and do not melt at ordinary temperature thus providing a suitable protective coating on skin and mucous membranes e.g. bismuth subnitrate iodoform paraffin paste (BIPP) and zinc paste.

h. **Patch:** Patch is a medicated adhesive strip or film that is applied to an external site to deliver active ingredients either to the outer surface of the body or into the body. Patch is sometimes also called 'extended release film' and is considered a specialized drug delivery system.

i. **Plaster:** Plaster is a preparation that adheres to skin and attaches to a dressing to afford protection and support, to furnish occlusion and macerating action, and/or to bring medication into close contact with the skin e.g. zinc oxide adhesive plaster and lidocaine medicated plaster.

j. **Poultice:** Poultice (Cataplasm) is a semi-solid preparation consisting of a soft heated mass of meal, clay, plant part or other substances that is spread on a cloth and applied to skin to treat an aching, inflamed or painful part or to improve circulation in localized area. It is commonly used on horses on lower legs to relieve inflammation and to treat easily-injured tendons in the area e.g. onion poultice and linseed (flaxseed) poultice.

k. **Sponge:** Sponge is a porous, interlacing, absorbent medicinal preparation typically used to cleanse a wound, to arrest bleeding or to apply a drug. Sponge usually retains its shape after use e.g. fibrinogen sponge and thrombin sponge.

l. **Suppository:** Suppository is a conical or ovoid solid preparation for insertion into body cavities other than mouth, where the solid inert base melts, dissolves or softens at body temperature and releases active ingredient to exert local or less often systemic effect. Suppository is identified by different terms according to the body cavity for which they are used viz. rectal suppository, vaginal suppository (sometimes also called pessary), nasal suppository and urethral suppository e.g. glycerine rectal suppository and bisacodyl rectal suppository.

3. Gases

a. **Spray:** Spray is a liquid preparation of drugs filled in a container under pressure and is released as an aerosol or mist on pressing a button. It is mostly used for local application to the naso-pharyngeal tract or the skin e.g. ipratropium nasal spray.

Special Types of Dosage Form

a. **Enteric coated tablet:** Enteric coated tablet is a special form of oral tablet preparation that controls the location in digestive tract where the drug is released and absorbed. The enteric-coated tablet is designed by coating the tablet with a material that dissolves in the relatively basic pH of intestine (pH > 5.5) but remains intact in the acid pH of stomach (pH ~3.5). Materials used for enteric coatings may include fatty acids, waxes and shellac. This preparation is primarily used for drugs which are destroyed by gastric acid (e.g. omeprazole) or which have an irritant effect on stomach (e.g. aspirin). It is also used for drugs those are intended to act in the intestine like anthelmintics and amoebicides.

b. **Sustained release capsule:** Sustained release capsule (Spanule) is a special type of oral capsule designed to release the drug gradually in the GI tract over an extended period of time. In this type, the finely powdered drug is first converted into pellets and the pellets are then treated with protective coatings of different thickness. These granules are then mixed thoroughly and filled into capsules. When ingested, the granules of different batches dissolve and release drug at different times so as to maintain constant drug concentrations in the blood stream (e.g. ibuprofen, indometacin and dextro-amfetamine sustained release capsules).

ROUTES OF DRUG ADMINISTRATION

The route of administration is the path by which a drug or any other substance comes in contact with or is introduced into animal's body. Selection of the route of drug administration is an essential factor in the therapy because it determines the onset and duration of action, and the effects produced by a drug. The pharmacokinetic properties of the drug such as absorption, distribution, metabolism and excretion are also critically influenced by the route of administration. Other factors which influence selection of route of drug administration include type and quantity of drug, number of animal (one or many) to be medicated, ease of administration, safety, and cost of the administration method. An improper selection of route may be either ineffective or harmful

to health of the animal. No single route of drug administration is ideal for all drugs in all circumstances.

Factors affecting route of drug administration

A number of factors should be considered in selection of route of administration of a drug. These factors may include:

a. **Physico-chemical properties of drug:** Drugs with high lipid solubility are more readily absorbed from all routes, including GI tract. In contrast, polar and ionised drugs (e.g. ouabain and heparin) are ineffective by oral route and are administered by parenteral routes.

b. **Formulation of drug:** Some drugs are insoluble in water and available as suspension or emulsion (e.g. procaine penicillin). Such drugs are not suitable for intravenous route.

c. **Nature of drug:** Peptides and acid labile drugs (e.g. insulin and penicillin-G) are rapidly destroyed in the GI tract. Therefore, such drugs are primarily administered by parenteral routes.

d. **Onset of action required:** Several routes (e.g. oral and subcutaneous) produce slow onset of action. Such routes are not suitable for emergency requirements.

e. **Type of response required:** Type of pharmacological or therapeutic effect for some drugs depends on the route of administration. For example, magnesium sulphate solution given intravenously produces muscle relaxation or paralysis, whereas when administered orally it induces purgation.

f. **Site of desired action:** Topical route is more suitable for localised conditions (e.g. eye drops in conjunctivitis), whereas parenteral routes are more useful for systemic and generalised effects.

g. **Biotransformation:** Some drugs have very short half-lives due to their very rapid biotransformation (e.g. dopamine and dobutamine). Such drugs are administered only by intravenous infusion.

h. **Condition of patient:** Oral route is not suitable for unconscious, uncooperative or vomiting patient. On the other hand, parenteral routes are less effective against gastrointestinal parasites.

Routes of Drug Administration

In human beings more than 100 routes of drug administration have been identified, but their number may be much lesser in animals. These routes of drug administration have been grouped in different ways by different agencies, sometimes with overlapping and confusing classification. However, the categorization of important routes of drug administration in animals into four groups viz. oral, parenteral, inhalation and topical appears to be most suited for veterinarians.

. Oral/Enteral Route

Oral (Per orem; Per os; P.O., PO) or Enteral (intestinal) route refers to administration of drugs by ingestion. It is the oldest, easiest and commonest mode of drug administration. In veterinary medicine, drugs are administered orally either in form of drenches or mixed in feed or water. Drugs may also be given through tubes placed in the gastrointestinal tract like nasogastric tube or stomach tube. After oral administration, the drug passes through the gastro-intestinal wall and travels to the liver before it is transported via the bloodstream to its target site. If a rapid effect is desired, the drug may be given before meals, as it is rapidly absorbed from an empty stomach. However if the drug is likely to cause gastrointestinal irritation, it is given with or immediately after meals. Depending on the properties of drug, both local (e.g. antacids and activated charcoal) and systemic effects (e.g. amfetamine and phenytoin) can be produced. Oral route is employed routinely in human and veterinary professions for administration of a variety of drug preparations including capsules, tables, suspensions, mixtures, powders, syrups and elixirs.

Advantages

a. It is the safest, least expensive and commonest route of drug administration.

b. It is most convenient for the patient, as no puncture or sterile procedures are necessary.

c. It does not require sterilization of drug preparation; however, proper hygiene must be maintained.

d. Both liquid and solid dosage forms can be administered conveniently.

e. It is most suitable route for gastrointestinal tract infections and parasites.

f. Relatively large volume of liquid drug preparation can be administered.

g. In case of drug sensitivity, the ingested drug can be removed from stomach either by applying gastric lavage or inducing emesis so as to prevent further absorption.

Disadvantages

a. It is not suitable for emergency situations and in critically ill patient due to slow (~30 – 90 min) and irregular absorption of drugs.

b. Drug absorption for many drugs is often irregular and unpredictable, and is influenced by ingesta and other GI disturbances.

c. It is not a suitable route for uncooperative, unconscious or vomiting patients. It is also not suitable for fractious, wild and zoo animals.

d. Some drugs (e.g. peptide and acid labile) are ineffective by oral route because they are destroyed rapidly in the stomach or rumen.

e. It is not a useful route for most drugs in ruminants because presence of large amount of ingesta in rumen hampers absorption of drugs.

f. Strong electrolyte or highly polar drugs are not absorbed by oral route, as they remain largely ionised in the GI tract.

g. Poor administration technique or ingestion may lead to intra-tracheal delivery and subsequent aspiratory bronchopneumonia.

h. It is not suitable for drugs, which undergo extensive intestinal or hepatic first-pass effects.

i. Long-term administration of some drugs may either cause damage to gastro-intestinal mucosa (e.g. aspirin) or destroy gut micro flora causing suprainfection (e.g. antibiotics).

j. It requires larger dose size as compared to parenteral routes.

II. Parenteral Routes/Injections

Parenteral (par = beyond, enteral = intestinal) literally means routes other than gastrointestinal (enteral), but technically it refers to drug administration by injections under one or more layers of skin or mucous membrane. Use of a needle attached with syringe or similar instrument is a pre-requisite for drug administration by all parenteral routes. The parenteral routes avoid first-pass effect, have rapid onset of action and are useful in uncooperative and fractious animals. Preparation of drugs for parenteral administration must be sterile and free of pathogens, and site of injection must be clean and disinfected. Most parenteral routes are employed for systemic effect of drugs, although some routes provide only local effect. The commonly used parenteral routes in veterinary medicine include intravenous, intramuscular, subcutaneous, intraperitoneal and epidural.

1. Intravenous route

In intravenous (IV, I.V.) administration, the drug solution is injected directly in one of the superficial veins. The aqueous solution containing the drug may be given either in a single dose (IV bolus, IV injection) or by continuous infusion (IV drips, IV infusion). For the intravenous bolus, a needle of appropriate size is inserted directly into a peripheral vein and the drug solution is administered either rapidly or gradually over 1 to 2 minutes. The drug immediately reaches systemic circulation and effects are produced rapidly. For infusion, the drug solution is moved by gravity from a glass bottle or collapsible plastic bag through thin flexible tubing to a catheter inserted in a peripheral vein. As a general rule, comparatively small amount of solution is given by intravenous injection and large amount is administered by intravenous infusion. An intravenous injection can be more difficult to administer than a subcutaneous or intramuscular injection, because inserting a needle or catheter into a vein requires special skill and proper restraining of animal. Intravenous route is frequently used for administering irritating solutions (e.g. anti-cancerous drugs), rehydrating fluids, total parenteral nutrition and whole blood.

Advantages

a. It produces quickest onset of action (15 – 30 sec.), as the drug directly released in vein reaches the heart immediately and is distributed throughout the body rapidly.
b. It is the best route to deliver a precise dose quickly and in a well-controlled manner throughout the body.
c. It is most reliable route for acute situations, emergency medication and intensive care therapy due to rapid action and predictable plasma drug concentration.
d. Drugs which are rapidly metabolised can be infused slowly over time to get a constant pharmacological effect.
e. It provides 100% bioavailability, as it by-passes the absorption phase and the whole dose is delivered to the blood (IV administration is absorption in itself).
f. It is the only route by which highly irritating and non-isotonic solutions can be given. This is because irritant solution administered by slow IV infusion is continuously diluted by circulating blood and also because veins are relatively insensitive to pain due to lesser nerve supply.
g. Large volume of fluids and large doses of drug can be administered without ill-effects by extending the time of infusion.

Disadvantages

a. It requires trained personnel and special skill for careful injection.
b. It is the most dangerous route of drug administration because it by-passes the most of body's defences exposing the vital organs to high concentrations of drug. Over dosage can lead to immediate toxicity.
c. It requires the drug preparation to be perfectly sterile, pure and soluble. Presence of even traces of un-dissolved particles or contamination may lead to life-threatening infection, abscesses or allergic reactions.
d. It is not suitable for insoluble drugs, oily substances, or drugs in suspension. Drugs must be in aqueous solution.
e. It is not suitable in circumstances where restraint of the patient is either not possible or not appropriate, which may occur with non-domestic and wild animals.
f. Poor injection technique or air embolism may put life of animal in danger.
g. Extravasation of irritant drugs may cause phlebitis and necrosis of adjoining tissues.
h. It provides short duration of action because drug is rapidly distributed, metabolised and excreted from body.
i. Use of blunt or large size needles may cause irreversible damage to veins.
j. Once injected, the drug cannot be removed.

2. Intramuscular route

In intramuscular (IM, I.M.) administration, the drug is injected deep between the layers of one of large skeletal muscles. Intramuscular administrations in animals are mostly carried out by hand injection, if circumstances allow, but may also be carried out by remote injection (darting). In some cases of non-domestic animals, use of a pole syringe (syringe attached to a long pole) may be considered. Skeletal muscle is more vascular but is less richly supplied with sensory nerves. Lipid-soluble drugs diffuse freely through capillary walls and are well absorbed when administered intramuscularly. Polar drugs of low molecular weight can also be absorbed rapidly, but those of high molecular weight are absorbed slowly mainly via the lymphatics. Further, depending on the type of formulation, the drug may be either absorbed fairly quickly (e.g. aqueous solution) or more gradually (e.g. suspension and depot preparation). Intramuscular route is used for providing only systemic effects of drugs. It may also be used for particular forms of medication, which are administered in small amounts. Intramuscular route is routinely used for administration of many vaccines, antibiotics and steroids.

Advantages

a. It can be used for solutions, aqueous and oleaginous suspensions, and other depot preparations.
b. It provides fairly rapid absorption of drugs (~10 – 20 min), except for oily, depot and sustained release preparations.
c. Mild to moderately irritant preparations can be injected, but are not generally advised as tissue reaction and necrosis may become evident with time.
d. It provides longer duration of drug action than IV injection, but usually a little shorter than for SC administration.
e. It is most suitable route for fractious, wild and zoo animals, which may be difficult to restraint.

Disadvantages

a. Large volume of fluids cannot be injected.
b. Improper injection technique may cause deposition of drug in nerves, blood vessels, fat or connective tissues.
c. Muscle damage may occur following injection of drugs; the amount of damage varies with the drug preparation injected. Repeated injections of some drugs may result in tissue necrosis.
d. Absorption is sometimes erratic, especially for poorly soluble drugs.
e. Improper pre-injection mixing of poorly soluble drug may lead to faster absorption of solvent causing precipitation of drug at the site.
f. It is not suitable for emergency situations because conditions like cardio-vascular collapse or shock may impede absorption of drugs.
g. It is contraindicated for conditions like thrombocytopenia and coagulopathy (bleeding tendency) as they may lead to hematomas.
h. Repeated use of same site may leave the muscles sore and stiff.

***Remote injection (Darting)*:** Remote injection is a special technique by which a drug is administered with the help of a gun or arrow to non-domesticated animals, both captive and free-living. The main advantage of darting is that it allows intramuscular injection at a distance without handling or restraining of the animal. The injection is made generally into the rump or back of hind leg of the animal; for larger species the neck or shoulder muscles may also be used. Limitations of remote injection include all the normal limitations for intramuscular injection plus risk of seriously hurting the animal. The animal may get hurt either due to the excessive impact force of the dart, hitting the dart at wrong tender sites of the animal, or the panic reaction of animal to the dart e.g. by running into an obstacle.

3. Subcutaneous route

In subcutaneous (SC, S.C., SQ) administration, the drug preparation is deposited in the loose subcutaneous tissue just under the skin. The injected drug moves from the subcutaneous tissue into capillaries and is then carried away by the bloodstream. Protein drugs which are large in size, such as insulin, usually reach the bloodstream through the lymphatic vessels because these drugs cannot easily enter into capillaries. The subcutaneous tissue is richly supplied by nerves but is less vascular. Therefore, subcutaneous route is more useful when slow and continuous absorption is required. It is used for providing systemic effect of drugs. The SC route is routinely used for administering many protein drugs, depot preparations and sustained release products. Certain hormonal drugs (e.g. progestogen) may be given by inserting plastic capsules (implants) under the skin.

Advantages

a. Relatively large volume of non-irritating drug may be administered.
b. Absorption of drugs is continuous but gradual and slower (~15 – 30 min) than from IM sites.
c. It is suitable for pellet implantation and depot preparations.
d. Rate of drug absorption can be manipulated to some degree by use of a vasoconstrictor or cold (to decrease rate) or application of heat or massage (to increase rate) to the injected site.

Disadvantages

a. It is not suitable for irritant drugs, as they may cause severe pain, tissue necrosis and sterile abscess formation.
b. It requires drug formulation to be strictly isotonic and at physiological pH.
c. It is not suitable in shock states because reduced peripheral circulation markedly hampers the absorption rate.

4. Intraperitoneal route

In intraperitoneal (IP, I.P.) administration, the drug preparation is deposited into the peritoneal space. The peritoneum provides a large surface area for absorption of drugs. Absorption of the drug from the injected site is via the portal system, so it is not useful for drugs those undergo hepatic first-pass metabolism. Peritoneal dialysis is becoming more frequently used in small animals with short-term renal failure or insufficiency.

Advantages

a. It provides fairly rapid absorption of drugs from large surface area.
b. It is suitable for administration of large volume of drugs.
c. It is suitable for small size laboratory animals (e.g. rodents) and neonates.

Disadvantages

a. It is not suitable for administering irritating compounds because they may induce chemical peritonitis.
b. It is not frequently used in large animals due to danger of infection and peritoneal adhesions.
c. Improper and faulty injection may lead to puncturing of intestine and other abdominal organs.

5. Intrathecal route

In intrathecal (intraspinal, subarachnoidal) administration, the drug is deposited into the subarachnoid space. These injections are made by inserting the needle through the vertebral interspinous spaces into the spinal fluid, usually in the lumber area. A small amount of local anaesthetic is often used to numb the injection site. Intrathecal administration is commonly used in human beings to produce spinal anaesthesia and for administering drugs to produce rapid and local effect on the brain, spinal cord and meninges. Local anaesthetics (e.g. bupivacaine), analgesics (e.g. morphine) and chemotherapeutic agents (e.g. methotrexate) are sometimes given by this route. It is rarely used in animals because the vertebrae in animals are more compressed (difficult to insert needle) and because animals are generally uncooperative for the smooth injection.

6. Intra-arterial route

In intra-arterial administration, drug is administered directly in an artery. It produces high concentration of drug in the dependent area for a short time, which occasionally is necessary in cancer chemotherapy and in special radiographic or other imaging techniques. Vasodilator drugs in the treatment of vasospasm and thrombolytic drugs for treatment of embolism are occasionally administered intra-arterially. Arterial injection can sometimes cause weakening of the artery wall (pseudoaneurysm) or fungal infection of the artery wall (mycotic aneurysm); both conditions can lead to life-threatening arterial bleeding.

7. Epidural route

In epidural administration, the drug is deposited through a vertebral interspace into the epidural space. In this route, needle does not penetrate the meninges and there is less risk of traumatic injury to the spinal cord. The preferred injection sites are between first and second coccygeal vertebrae in horses and cattle. In veterinary medicine, epidural injection is commonly used in large animals to produce epidural anaesthesia (see also epidural anaesthesia, Chapter 26).

8. Intramedullary/Intraosseous route

In intramedullary administration, drug is administered directly in the marrow of the bone. The needle is injected right through the bone and into the soft marrow interior. It is used mainly for blood transfusion or fluid therapy when other routes (e.g. intravenous) are not possible, as in neonates.

9. Intradermal/Intracutaneous route

In intradermal administration, drug is injected within the layers of skin (dermis). This route may provide localised effect and is used mainly for testing the hypersensitivity of drugs or producing infiltration anaesthesia. It is also used for skin testing some allergens and for mantoux test for Tuberculosis. Occasionally, it is used for vaccination purposes.

10. Intra-articular route

In intra-articular administration, drug is administered directly into the joint space. It is used primarily to obtain high local concentration of drugs within the inflamed joint, without much danger of systemic toxicity. It requires strict aseptic conditions.

11. Intracardiac route

In intracardiac administration, drug is administered directly into the heart muscle. It is rarely used in emergency situations, especially during cardiac arrest. Epinephrine is occasionally administered by this route during cardiopulmonary resuscitation.

12. Intratracheal route

In intratracheal administration, drug is administered in between the rings of trachea, especially in the middle third of the neck. It is used primarily for treatment of respiratory tract infections.

13. Intracerebral

In intracerebral administration, the drug is injected directly into the brain. It is used mainly in human medicine in the treatment of malignancies of the brain. It is also used in experimental research for testing effect of drugs on brain.

14. Other parenteral routes

Some other parenteral routes used for administration of drugs for specific purposes include intraruminal route (into the rumen), intrapulmonary route (into the lungs) intravesical route (into the urinary bladder), intracavemosal (into the base of penis) and intravitreal route (through the eye).

III. Inhalation/Pulmonary Administration

In inhalation or pulmonary administration, drugs are given through breathing via nasal passage. Some drugs are also inhaled through the mouth. Drugs administered by inhalation are generally given either by atomization into smaller particles or as gases so that the drug can easily pass through trachea into the lungs. Once inside the lungs, drug is absorbed from alveoli into the bloodstream and distributed rapidly throughout the body. Penetration into the lungs and further absorption largely depends on the physico-chemical properties of drug and the size of droplets. The lipid soluble volatile liquid substances and gases (e.g. halothane and nitrous oxide) are rapidly absorbed from alveoli and produce mainly systemic effects, whereas the non-volatile, polar or ionised substances (e.g. ipratropium), mainly administered as aerosols, remain largely unabsorbed in respiratory tract and produce local effects. Similarly, smaller droplets go deeper inside the alveoli and are better absorbed to produce systemic effect, but the large droplets which are retained in the upper respiratory tract have mainly local effects. As a general rule, very large drug particles (> 20 micron) do not enter respiratory tract, large drug particles (5-10 micron) are deposited in the upper airways, intermediate-sized particles (3-5 micron) go further and get deposited in the smaller airways of bronchi and bronchioles, small particles (3 micron) behave more like gas molecules to reach and get deposited in the alveoli and very small particles (< 0.5 micron) reach alveoli but fail to get deposited there as they are exhaled back.

Drugs given by inhalation include various preparations for respiratory infections and diseases, medicinal gases such as oxygen, and inhalation anaesthetics. Delivery of drugs into the respiratory passages is generally done by standard techniques or machines like anaesthetic machines in case of inhalation anaesthetics, and aerosol producing devices like vaporisers, atomisers, nebulisers, inhalers, etc. for non-volatile substances. Inhalation of aqueous preparations such as medicated steam and sprays are also used. Oily preparations are not given by inhalation since the oil damages pulmonary tissue.

Advantages

a. It provides rapid onset (~2 – 3 min) of systemic action for volatile liquids and gases because the alveoli offer highly vascular thin mucous membrane and vast surface for drug absorption.
b. It provides controlled administration of drug for moment-to-moment adjustment.
c. It avoids drug loss through hepatic- and intestinal- first pass effects.
d. It is suitable for respiratory tract diseases where many drugs remain unabsorbed and have only local effect.

Disadvantages

a. It generally requires special techniques or devices.
b. It is not suitable for irritant vapour and gases because such drugs cause irritation and inflammation of respiratory tract.
c. It is difficult to regulate the exact amount of dosage.
d. It is not a convenient route for domestic animals.

IV. Topical Administration/Local Application

Topical administration refers to application of drugs to body surfaces like skin and mucous membrane. The drug administration by this route generally affects the part of body to which it is applied thus producing localised effect. However, some drugs applied topically are absorbed from the site of application and produce systemic effect.

Skin

A large number of drugs are applied externally on skin to produce variable pharmacological effects either on the skin or deep inside the skin. Depending on the site of drug action and penetration through skin, the route of application could be called epicutaneous (outside skin) or percutaneous (through skin). The epicutaneous application is primarily meant to deliver active principles only to the site of application to produce localized effect. In the percutaneous application (often called transcutaneous

or transdermal route), drugs are specially designed to cross the intact skin to produce systemic effects. Systemic effects in animals may also occur when drugs applied epicutaneously either are absorbed through the damaged or inflamed skin or are licked by the animal. Absorption of the drug through skin generally occurs in proportional to its lipid solubility because the epidermis often behaves like a lipid barrier. Use of occlusive dressings, inunctions, or special drug delivery devices like transdermal patches generally enhances absorption through skin to produce systemic effects. Epicutaneous route is commonly used to eradicate ectoparasites and to treat superficial skin disorders such as psoriasis, eczema, dermal infections (viral, bacterial and fungal), itching and photosensitization. Certain opioids (e.g. fentanyl) used in pain therapy are specially designed as transdermal patches to deliver the active constituent percutaneously (See for detail transdermal delivery systems). The drug preparations meant for application on skin include ointments, lotions, liniments, solutions, creams, powders and gels. The application of drugs on skin may be done in different ways; selection of which depends on factors such as type and formulation of drug, species and size of animal, type of action required and cost.

a. **Bath:** Bath is bathing or immersion of the body or any of its parts in medicated water or any other fluid medium. Baths are termed 'general' when whole body is bathed/ immersed in the fluid and 'local' when certain parts are dealt with (e.g. foot bath). Baths are given generally for their local effect upon the skin in cutaneous disorders.

b. **Dip:** Dip is a type of bath in which the whole body is immersed for a time in medicated fluid, particularly insecticidal fluid. It ensures complete wetting of the animal in the medicated fluid and is more useful in small to medium sized animals like sheep and goats. In veterinary medicine, dips are generally used to kill ectoparasites in small animals.

c. **Inunction:** Inunction is the application of a semisolid or liquid drug preparation on the surface of body by rubbing or friction. Inunction is generally used for application of ointments, liniments, oils, etc. for their local action, or occasionally with purpose of causing absorption of the active ingredient.

d. **Dusting:** Dusting in the application of fine particles of solid matter on the surface of body. It is used in therapeutics for application of dusting powders on skin for superficial skin conditions or in body cavities for surgical conditions. Insecticidal powders are commonly applied on body surface by dusting for eradication of external parasites.

e. **Spray:** Spray is the application of drug on skin in the form of a mist or cloud of small flying drops produced by a spray machine. It is a useful method of drug application in almost all species of animals. In small animals, coat should be combed or brushed in opposite direction and spray is applied to roots of the fur. Spray is mostly used for application of analgesics, counter-irritants and ectoparasiticides.

f. **Pour-on:** Pour-on is the application of a drug solution, particularly an ectoparasiticide solution, along the animal's dorsal midline behind the shoulders to the hipbones. In some cases, it is applied to the top of head and around the base of horns. In pour-on application, some of the drug is absorbed percutaneously and produces systemic action. Pour-on application is commonly used for the control of horn flies, lice and other arthropods of cattle and sheep.

g. **Spot-on:** Spot-on application is similar to pour-on, but in this method the specified amount of drug solution is applied to a small area on the head or back. Spot-on treatments are commonly used for application of ectoparasiticides in almost all species.

Mucous Membranes

Topical application of drugs on mucous membranes is routinely used for providing localised action. In such cases, the drug remains largely unabsorbed at the site of application. Some drug preparations have been developed recently even to provide systemic effect through mucous membranes (called transmucosal route). Drug absorption through a mucosal surface is generally more efficient than skin because the stratum corneum epidermidis, the major barrier to transdermal absorption, is absent. Mucosal surfaces are also rich in blood supply and avoid, in most cases, degradation by first-pass hepatic metabolism. The amount of drug absorbed through mucous membrane depends on several factors such as drug concentration, size of drug molecule, drug lipid solubility, type of formulation, mucosal contact time, pH of absorption site and venous drainage of mucosal tissues.

a. **Sublingual and buccal routes:** Sublingual (below the tongue) and buccal (spread over buccal mucosa or held in mouth) routes are useful for highly lipid soluble and non-irritating drugs. The thin epithelium and the rich capillary network under the tongue or in buccal mucosa permit rapid drug absorption and pharmacological action. Additionally, the drug is saved from destruction from digestive enzymes and hepatic inactivation as it reaches the general circulation without traversing through GI mucosa and liver. The drugs are specially formulated for these routes in the form of either soft tablets for faster dispersal and absorption (sublingual route) or hard tablets for relatively slower release and absorption (buccal route). These routes are primarily employed in human beings for administration of some drugs, which undergo extensive intestinal or hepatic first pass effect and for which rapid action is required. For example, the sublingual route is employed for nitroglycerine administration to relieve angina pain and nicotine containing chewing gum is used by buccal route for cigarette smoking cessation. Both routes are not much suitable for animals because ingestion of drugs by animal often decreases their efficacy. Further holding of tablet in mouth is inconvenient and relatively impractical in animals.

b. **Rectal/Per-rectal route:** Rectal administration refers to insertion of drugs into the rectum either in the form of a suppository or as an enema. Rectal suppositories containing drugs are primarily used for laxative purposes and for providing systemic effect in specific conditions where it is undesirable or impossible to deliver the medication by mouth, such as when a patient is experiencing intractable vomiting or having engulfing problems. The main use of enemas is as laxatives or to

clean the lower bowel prior to examination or surgery. Enema may also be used for administration of drugs into the bloodstream. The rate of drug absorption from rectum is generally slow, incomplete and erratic. However for some drugs it is quite useful as some of the veins draining the rectum lead directly to the general circulation, thus by-passing the liver. In animals, rectal route is largely used to produce local effects by enema (e.g. soap water enema) or suppository (e.g. bisacodyl suppository), and in rare cases for providing systemic effects (e.g. chlorpromazine).

c. **Intraocular route:** In intraocular administration, the drugs are applied topically as drops, solution, gel or ointment onto the conjunctiva. Solid inserts, which release the drug continuously and in slow amounts, are also available, but they may be hard to put in and keep in place. Topically applied ophthalmic drugs are almost always used for their local effects in the eye. Occasionally, systemic absorption may result from drainage through the nasolacrimal canal. Intraocular route is frequently used to treat several eye disorders including application

Table 3.1: Important features of selected routes of drug administration.

Route	Site of administration/application	Effect	Primary dosage form(s)
Oral	Mouth (Ingestion)	Systemic, local	Bolus, capsule, tablet, solution, suspension, mixture, syrup, paste, powder, granule, controlled release products.
. Parenteral			
1. Intravenous	Peripheral vein (H, C, G, S = jugular; D, C = cephalic and recurrent tarsal; P = ear)	Systemic	Solution (aqueous).
2. Intramuscular	Skeletal muscle (gluteal muscle)	Systemic	Solution, suspension, depot preparations.
3. Subcutaneous	Subcutaneous tissue (H, C = middle third of neck; D = inner aspect of thigh).	Systemic	Solution, suspension, implant, depot preparations.
4. Intraperitoneal	Peritoneal space	Systemic	Solution.
5. Intrathecal	Subarachnoid space (spinal)	Local (lumbar region)	Solution.
6. Epidural	Epidural space (H, C = between 1st and 2nd coccygeal vertebrae; D = between last lumbar and first sacral vertebrae)	Local	Solution.
7. Intradermal	Within layers of skin	Local	Solution.
II. Inhalation (Pulmonary)	Respiratory tract (inhalation)	Local, systemic	Gas, volatile and non-volatile solutions.
V Topical			
Skin			
. Bath	Skin surface	Local	Solution, emulsion.
. Dip	Skin surface	Local	Solution, emulsion.
. Inunction	Skin surface	Local, systemic	Cream, paste, oil, plaster, lotion, spray, solution, ointment.
. Dusting	Skin. body cavity	Local	Dusting powder.
Mucous membrane			
. Sublingual and buccal	Below tongue, buccal mucosa	Local, systemic	Solution and tablet.
. Rectal	Rectum	Local, systemic	Solution, ointment, suppository.
. Intraocular	Eye	Local	Solution, suspension, ointment.
. Intranasal	Nose	Local, systemic	Solution, ointment, sprays, inhalant, snuff.
. Intramammary	Teats	Local	Ointment, solution.
. Intravaginal	Vagina	Local	Solution, ointment, suppository, douche, tablet.
. Intrauterine	Uterus	Local	Solution, ointment, douche, suppository, tablet.
. Intraurethral	Urethera	Local	Solution, suppository.
. Intra-aural/aural	Ear	Local	Solution, suspension.

Abbreviations: D = dogs; C = cattle; G = goats; H = horses; P = pigs; S = sheep.

of artificial tears to relieve dry eyes, mydriatics to dilate pupil, anti-glaucoma drugs (e.g. pilocarpine) to lower intraocular pressure and antibiotics (e.g. chloramphenicol eye drops) for conjunctivitis.

d. **Intranasal route:** Nasal mucosa is often treated with drug solutions applied as nasal sprays or drops. Topical application in nose is occasionally used to produce systemic effects also (e.g. desmopressin and buscrelin) because nasal mucosa is highly vascular and offer good absorptive surface. Drugs administered by this route generally have rapid onset of action. Intranasal route is commonly used to administer decongestants for rhinorrhoea and corticosteroids for allergies. In human medicine, oxytocin, vasopressin and polypeptide hormones from posterior pituitary are sometimes administered intranasally as a snuff.

e. **Intramammary route:** In intramammary route, drugs are administered into the orifice of a teat usually with help of a teat siphon. Absorption tends to be more limited following intramammary administration, but it may vary with the pathological state of the udder. Intramammary route is commonly employed in milch animals for treatment of mastitis.

f. **Intravaginal route:** In intravaginal route, drugs are applied topically on the vagina to provide local action. A few drugs may also be slowly absorbed from vaginal mucous membrane to have some systemic action. This route is often used in female animals and women to treat a variety of gynaecological disorders. Various drug preparations including solutions, tablets, creams, gels and suppositories are available for vaginal application.

g. **Other routes:** Several other routes which may be used for topical application of drugs on mucous membranes include intrauterine, intraurethral, prepucial and aural (ear). Important features related to these routes are given in Table 3.1.

Specialised Drug Delivery Systems

In recent years, several specialized drug delivery systems/ devices have been developed to deliver the drug specifically to site of action (targeted delivery), to avoid fluctuation in plasma drug levels (controlled release), to achieve greater efficacy, to overcome cellular barrier and enzymatic degradation or to minimize toxic effects. Various specialized drug systems or formulations are available for application on skin (e.g. transdermal delivery systems), parenteral injections (e.g. needle free injection) and administration by oral route (e.g. sustained release capsule and enteric coated tablet). Some other drug delivery systems, such as liposomes, microparticles and nanoparticles are highly selective in the release of drug to the target site.

1. Transdermal drug delivery systems (TDDS)

Transdermal drug delivery system (TDDS) mainly comprises of a medicated adhesive patch that delivers the contained drug(s) at a predetermined and controlled rate into systemic circulation via the stratum corneum. Transdermal drug application significantly alters the drug kinetics and dynamics with gradual and continued absorption, enhanced bioavailability, longer duration of action and minimal side effects. The TDDS bypasses the first-pass effect and is considered useful for a drug having either very short half-life or narrow therapeutic index. The adhesive patch can be removed if side effects appear. The adhesive patches of various sizes and shapes are available for delivery of various drugs in man and animals. In veterinary medicine, transdermal patches are used primarily in dogs and cats to deliver drugs like opioids (see for detail Fentanyl transdermal patch, Chapter 24). In human medicine, these are used to deliver a variety of pharmaceuticals such as opioids (in pain therapy), nicotine (for cigarette smoking cessation), nitroglycerine (for angina pain), hormones (in contraceptives programme), hyoscine (for motion sickness), anti-depressants (for depression) and clonidine (for hypertension).

2. Needleless injection system/Dermojet

Dermojet, a patented product manufactured and distributed by M/s Robbins Instruments Inc., is a special type of syringe in which needle is not used. In this method of subcutaneous administration, a highly compressed drug solution is stored in a gun like device and released as a high velocity jet from a micro fine orifice by pressing a button. The released liquid rapidly passes through the skin layers and gets deposited in the subcutaneous tissue. The subcutaneous injection is confirmed by formation of a large characteristic wheal with clearly visible center of circumscribed area where the liquid penetrated the skin. The depth of the injection can be adjusted by changing the distance and angle of the appliance in relation to the cutaneous surface. Similar to a gun, the injection can be reloaded, one after another, in a very short time simply by lowering the lever. The dermojet method is painless and suited for mass inoculations. It can be used for administration of anaesthetic solutions, parenteral drugs, vaccines and steroids in aqueous suspension. The dermojet is not recommended to be used in any area likely to undergo lesions following the injection under pressure such as the eyeball and blood vessels.

3. Liposomes

Liposomes (meaning lipid bodies) are minute vesicles consisting of one or more encapsulated compartments formed by one or more than one phospholipid bilayers. The polar and water soluble drug molecules are incorporated in the aqueous compartment of liposome and the amphiphilic and lipophilic molecules are solubilised within the phospholipid bilayer according to their affinity towards the phospholipids. Liposomes are available in different sizes (25 to 5000 nm) and are administered by parenteral routes. After administration, the liposomal carrier system takes the drug across cell membranes to the target sites or areas not normally accessible to the free form of the drug. The composition of liposomes also facilitates drug delivery into reticuloendothelial cells because these cells phagocytose liposomes and thus the enclosed drug. The major application of liposomes is their capacity to deliver and internalise a variety of drugs (e.g. antineoplastics, immunomodulators and antibiotics), proteins/peptides (e.g. human interleukin-2, antibodies), vaccines and genes for disorders at the specific target sites. Liposomes themselves are biodegradable, non-toxic and immunologically inert.

4. Aerosol therapy

Aerosol therapy is the administration of drugs in the form of aerosols directly to the respiratory tract. Because the drugs are deposited locally in the airways, aerosol therapy allows lower dosage, provides rapid action and minimizes incidence of side effects and systemic toxicity. The drugs commonly employed by this technique include bronchodilators and anti-inflammatory agents used for obstructive airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Aerosol therapy is commonly used in human medicine and has also some application in animals, particularly horses.

Devices used for aerosol therapy: The devices available for generation of aerosols can be broadly divided into three categories: nebulizers, metered dose inhalers and dry powder inhalers. Each delivery system has unique deposition characteristics which should be considered before administration of drugs.

a. Nebulizers: A nebulizer is a device used to administer a liquid medication in the form of a mist inhaled into the lungs. The common technical principal for all nebulizers is to either use oxygen/compressed air or ultrasonic power, as means to break up medicated solutions or suspensions into small aerosol droplets, for direct inhalation from the mouthpiece of the device. Depending on the technique used, two types of nebulizers are available: jet nebulizer and ultrasonic nebulizer. The jet nebulizer, also called atomizer, uses a powerful compressor to generate high air flows across the liquid medication to produce small diameter aerosol particles. The size of aerosol particles and hence their pulmonary distribution can be changed by altering the gas flow rate. The jet nebulizers are cheap, but the compressor produces noise, which disturbs animals. The ultrasonic nebulizer, in contrast, uses an electronic oscillator to generate a high frequency ultrasonic wave, which causes mechanical vibration of a piezoelectric element. This vibrating element is in contact with a liquid reservoir and its high frequency vibration produce an aerosol. The ultrasonic nebulizers can produce highly concentrated aerosols and are relatively silent, but they are very costly. Additionally, the diameter of generated particles is greater than those obtained using jet nebulizers at high air flow rates. Some types of nebulizer have been adapted for equine use.

b. Metered-dose Inhalers (MDI): The MDI is a device that delivers an accurate dose of a drug through a metering valve during inspiration. A metered-dose inhaler consists of three major components: a canister or container where the drug is either dissolved or suspended in the propellant resides, a metering valve that allows a metered quantity of the formulation to be dispensed with each actuation and an actuator (or mouthpiece) which allows operating the device and directing the aerosol into the patient's lungs. Adaptation of MDI to use in horses has led to the development of several systems.

c. Dry powder inhalers (DPI): The DPI is a breath-actuated device that delivers drug(s) to the lungs in the form of a dry powder. The medication is commonly held either in a capsule for manual loading or a proprietary form from inside the inhaler. Once loaded or actuated, the operator puts the mouthpiece of the inhaler into the patient's mouth and allows it takes a deep inhalation. The dose that can be delivered in a single breath is typically less since larger powder doses may lead to provocation of cough. The adaptation of the DPI for use in the horse necessitates an airtight face mask.

Fixed Dose Combination Preparations

Fixed dose combination (FDC) preparations are combination products of two or more active drugs, often in a fixed-dose ratio available in a single dosage form. A large number of pharmaceutical preparations including vitamins, minerals, antitussives, anti-diarrhoeals, antacids, analgesics and tonics contain two or more drugs and are widely used in both human and veterinary practices. FDCs are also available for the treatment of various diseases ranging from bacterial infections to cardiovascular diseases. Fixed dose combinations offer some advantages, but they also have few limitations. They are valuable only when they have been developed based on sound pharmacokinetic and pharmacodynamic criteria. Combinations should only be used if each component is necessary for the desired effect and if the advantages outweigh the added risks of using 2 or more drugs. Therefore before prescribing a combination the practitioner must consider whether all drugs in the preparation are essentially required or if one drug alone would be adequate.

Advantages

a. They are more convenient to administer to animals because all required drugs could be given at a single time.
b. They increase the therapeutic efficacy when combination drugs have synergistic (e.g. sulphamethoxazole + trimethoprim) or potentiative (levodopa + carbidopa) effect.
c. They reduce the administration cost due to single packaging and fewer prescriptions.
d. They reduce chances of development of drug-resistant strains in case of infectious diseases.
e. They decrease chances of adverse effects as side effect of one drug may be counteracted by the other drug (e.g. furosemide + spironolactone).

Disadvantages

a. They are not useful when animal requires action of only one drug. In such conditions, administration of FDC will only enhance the cost and possibly the adverse effects.
b. They are not successful when different drugs in the preparation have variable pharmacokinetics.
c. They do not allow alteration in the dose of one drug without affecting the dose of other drug.
d. In case of adverse reactions, the adverse effect of the preparation cannot be ascribed to a particular drug.
e. Contraindications to one drug (e.g. hypersensitivity) may contraindicate the whole preparation.
f. Some drugs when combined lead to increased toxicity (e.g. the anti-TB drugs streptomycin, kanamycin and capremycin when combined produce enhanced oto- and nephro-toxicity).

g. They encourage polypharmacy allowing pharmaceutical firms to adopt unethical practices.

Polypharmacy: Polypharmacy means administration of multiple drugs at single time, even when all are clinically not required. Polypharmacy is different from fixed drug combinations in which a single preparation contains two or more drugs and in which two or more drugs are always administered as a fixed dose for a combined effect. Polypharmacy is most commonly observed in elderly humans, but is also widespread in veterinary practice. The most common limitations of polypharmacy are increased adverse drug reactions, drug-drug interactions, high costs and poor compliance with drug therapy. It also beats the purpose of rational therapeutics when selected drugs are recommended for specific conditions. If often encourages off-label use of drugs.

Off-label use: Off-label use is the practice of prescribing drugs for conditions for which they have not been approved. Off-label use of medications is legally permitted in many countries and is a common practice in both human and veterinary medicines. Examples of off-label use of drugs in human practice may include prescribing tricyclic antidepressants to treat neuropathic pain and magnesium sulphate to treat premature labour and preeclampsia. Use of human drugs in animals also constitutes the off-label use and is a very common practice in veterinary therapeutics.

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CHAPTER 4 PHARMACOKINETICS

- Passage of drugs across biological membranes
  - Simple transport
    - Diffusion
    - Filtration
  - Specialized transport
    - Active transport
    - Facilitated diffusion
    - Pinocytosis
- Absorption
  - Gastrointestinal tract
  - Parenteral sites
  - Pulmonary sites
  - Topical sites
- Distribution
  - Physicochemical properties of drug
  - Binding to plasma and tissue proteins
  - Blood flow and organ size
  - Specialized compartments and barriers
  - Availability of specialized transport system
  - Disease states
  - Physiological factors
- Metabolism/Biotransformation
  - Functions of metabolism
  - Sites of metabolism
  - Drug metabolising enzymes
    - Microsomal enzymes
    - Non-microsomal enzymes
  - Pathways of biotransformation
    - Phase I Reactions
      - Oxidation
      - Reduction
      - Hydrolysis
    - Phase II Reactions
      - Conjugation with glucuronic acid
      - Conjugation with sulphate
      - Conjugation with methyl group
      - Conjugation with glutathione
      - Conjugation with acetyl group
      - Conjugation with amino acids
      - Conjugation with thiosulphate
  - Induction of drug metabolising enzymes
  - Inhibition of drug metabolising enzymes
- Excretion
  - Renal excretion of drugs
  - Extra-renal excretion of drugs
    - Biliary excretion
    - Pulmonary excretion
    - Mammary excretion
    - Salivary excretion
    - Gastro-intestinal excretion
    - Other routes of excretion

Pharmacokinetics is a branch of pharmacology that deals with the study of absorption, distribution, metabolism and excretion of drugs. The word pharmacokinetics is derived from Greek words *pharmakon* (drug) and *kinesis* (movement), meaning the movement of a drug. Accordingly, in simple words, pharmacokinetics is the study of drug movement in (absorption), through (distribution) and out (metabolism and excretion) of the body. The study of pharmacokinetics is essential in therapeutics because it determines the route of administration, dose, onset of action, time of peak effect, duration of action and frequency of drug administration.

Drugs and Cell Membrane

Drugs are administered into body with the aim of producing their desired effect on the target organs. Once a drug is administered at an appropriate site, it must be absorbed effectively into the blood stream, distributed efficiently to site of action, and subsequently metabolised and excreted from the body (Figure 4.1). The processes of absorption and distribution are responsible for placement or deployment of the drug in body, and metabolism and excretion for elimination of the drug from body. The term

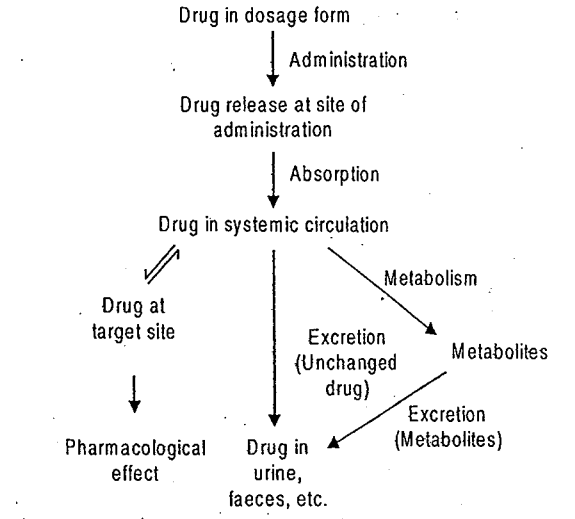


Fig. 4.1. Schematic representation of drug administration and pharmacokinetic processes.

'drug disposition' is often used to describe the simultaneous effects of the processes of drug distribution and elimination (metabolism and excretion), usually after IV administration. All pharmacokinetic processes involve movement of drugs across biological membranes.

Biological membranes

Biological membranes are selective molecular barriers to the body environment, which segregate organ function, separate cells from one another, and compartmentalise intracellular events. Regardless of the route of administration, the drug must cross a membrane(s) before it reaches its site of action. The composition of membrane barriers is highly variable i.e. they may be composed of several layers of cells (e.g. skin, vagina and placenta), a single layer of cell (e.g. enterocytes and renal tubular epithelial cells), or even less than one cell in thickness (single cell membranes, nucleus and mitochondria). The matrix of the biological membrane is composed of polar lipid molecules (e.g. phospholipids) arranged in a bilayer with their hydrophobic tails (fatty acid chains) extended inward and hydrophilic heads aligned at both surfaces (Figure 4.2). The bimolecular layer of lipid molecules is mainly responsible for regulating the passage of drugs into and out of the cell. Membranes also contain proteins, which are embedded in the lipid bilayer either completely or partially, and some carbohydrates those are attached to either the membrane proteins or lipids. The cell membranes also have small pores through which filtration of molecules can take place. Paracellular spaces or channels also exist between certain epithelial/endothelial cells.

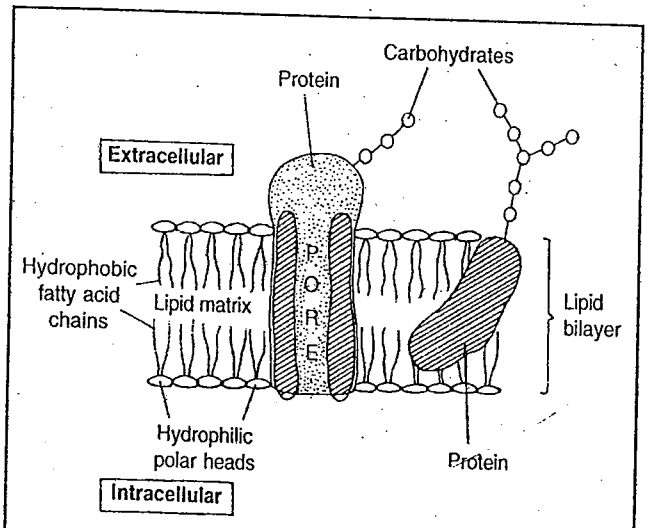


Fig. 4.2. Arrangement of biological membrane.

PASSAGE OF DRUGS ACROSS BIOLOGICAL MEMBRANES

Drugs and other substances cross biological membranes by several processes. These transport processes have been broadly divided into two groups, simple transfer and specialised transport. The simple transfer consists of diffusion and filtration, while the specialised transport system consists of active transport, facilitated transport and pinocytosis. Passive transfer or simple diffusion is the most important process for xenobiotics, although specialized transport systems are also used for a limited number of therapeutic agents.

I. Simple Transport

1. Diffusion

Diffusion (Simple diffusion or passive diffusion) is the mo important means of drug passage across the biological membran In diffusion, the drug present in aqueous medium at one end c the biological membrane partitions and dissolves in lipid phase the membrane and finally leaves it by dissolving again in aqueou medium at the other side of the membrane. The simple diffusi is meant for lipid soluble substances only because they dissolv rapidly in the lipoid matrix of the membranes and, thus, diffu quickly. The unionised (undissociated) drugs also penetrat biological membranes by diffusion because the unionised forn of the drug are generally much more lipid soluble than thei ionised form. (See also exceptions in the present Chapter).

Simple diffusion is an energy independent and non-saturabl process. It does not involve a carrier and shows a low structura specificity. The driving force for diffusion of a drug is th concentration gradient across a biological membrane separating two body compartments. Accordingly the drug molecules alway diffuse from a region of higher concentration to one of the lower concentration (along concentration gradient or downhill movement until equilibrium (when drug concentration on each side of the membrane becomes equal) is attained. Drugs those exist in bod in both ionised and unionised forms approach equilibrium by transfer of unionised form only. For a drug to be readily diffusible it mus be largely lipid soluble, yet it must have some solubility in aqueous solution also. In other words, a perfect lipophilic-hydrophilic balanc (LHB) in the structure of the drug should be maintained for undergoing appropriate diffusion. Water-soluble drugs penetrat the cell membrane poorly as they cannot cross the lipoidal membranes. Extremely lipid soluble drugs are also poorly diffusibl because they are totally insoluble in aqueous body fluids and, therefore, cannot gain access to the surface of cell. In addition t the essential requirements mentioned above, the passive diffusion is affected to variable extents by the molecular weight of drug, thickness of membrane and surface area for diffusion. Drugs having molecular weights between 100 and 400 Da effectively cross biological membranes by diffusion, but those with high molecular weight undergo poor diffusion. Large surface areas (e.g. intestine and lung) and thin membranes favour the process of diffusion. The passive diffusion of drug molecules across a biological membrane taking into account the concentration gradient, diffusion co-efficient, lipid solubility, surface area and thickness of membrane is best expressed by Fick's first law of diffusion:

Rate of diffusion (molecules per unit time)

$$= (C_1 - C_2)\ \frac{A \times D \times K_{m/w}}{T}$$

where C_1 is the higher concentration and C_2 is the lower concentration and $C_1 - C_2$ is the difference in the concentrations of drug in two media across the biological membrane called the concentration gradient; A is the surface area of the membrane across which diffusion is occurring; D is diffusion coefficient of drug through the membrane; $K_{m/w}$ is partition coefficient of drug in the lipoidal membrane and the aqueous fluid (lipid: water partition coefficient); and T is the thickness of the membrane.

The pH-partition hypothesis: Most drugs are weak acids or weak bases and exist in physiological solutions in both ionised and unionised forms. The amount of drug that exists in unionised

form or ionised form is a function of its pK_a (negative logarithm of dissociation constant) and the pH of the fluid in which it is dissolved. When the pH of the fluid is same as the pK_a value of the drug, the ratio of unionised to ionised forms is 1:1 (therefore, pK_a is numerically equal to the pH at which drug is 50% ionised). As the pK_a value is a characteristic of the drug (pK_a value is a constant for an acid or a base), the degree of its ionisation depends primarily on the pH of the medium. The relative amount of ionised and unionised drug in the body fluid at a particular pH and the per cent of drug ionised at this pH can be determined by Henderson-Hasselbalch equation:

For weak acidic drug

$$pK_a - pH = \log \frac{\text{Concentration of unionised drug}}{\text{Concentration of ionised drug}}$$

$$\% \text{ Ionised drug} = \frac{100}{1 + \text{antilog}\,(pK_a - pH)}$$

For weak basic drug

$$pH - pK_a = \log \frac{\text{Concentration of unionised drug}}{\text{Concentration of ionised drug}}$$

$$\% \text{ Ionised drug} = \frac{100}{1 + \text{antilog}\,(pH - pK_a)}$$

Exceptions: There are few exceptions to the basic principles mentioned above:

a. Strong acids (e.g disodium cromoglycate) and strong bases (e.g. mecamylamine) remain ionised at all body pH. In contrast, very weak acids (e.g. pentobarbital) and very weak bases (e.g. acetanilide) largely exist in unionised forms at all body pH values (Table 4.1).

b. Some drugs are highly water soluble in both ionised and unionised forms (e.g. penicillin and streptomycin) and are poorly diffusible. The insufficient lipid solubility of the unionised form of aminoglycoside antibiotics is mainly due to the preponderance of hydrogen-bonding groups such as –OH in their sugar moiety.

Table 4.1: pK_a values of some acidic and basic drugs.

Acidic Drug	pK_a	Basic Drug	pK_a
Very weak acids (pK_a >8.0)		*Very weak bases (pK_a <5.0)*	
Pentobarbital	8.1	Acetanilide	0.5
Hexobarbital	8.2	Antipyrine	1.5
Phenytoin	8.3	Benzocaine	2.5
Phenolphthalein	9.7	Diazepam	3.4
Ascorbic acid	11.5	Quinidine	4.4
Weak acids (pK_a 2.5 – 8)		*Weak bases (pK_a 5.0 – 11.0)*	
Cloxacillin	2.8	Reserpine	6.6
Enalapril	3.0	Trimethoprim	6.4
Aspirin	3.5	Diltiazem	7.7
Ibuprofen	4.4	Verapamil	8.9
Thiopental	7.5	Atropine	9.7
Strong acids (pK_a <2.5)		*Strong bases (pK_a >11.0)*	
Disodium cromoglycate	1.9	Mecamylamine	11.2
Cefazolin	2.1	Neostigmine	12.0

c. Some drugs (e.g. digoxin) are incapable of becoming ionised at any pH in body. These are non-polar drugs which diffuse readily across the membranes.

d. Some acidic (e.g. heparin) and basic (e.g. ipratropium and quaternary ammoniums) are permanently ionised at all physiological pH. They are polar drugs and are non-diffusible.

e. Ionised forms of some drugs also undergo diffusion to a considerable extent due the presence of large lipophilic group(s) in their structure (e.g. morphinan derivatives).

f. Some drugs (e.g. epinephrine and ampicillin) can yield both anions and cations; so they behave like both acids and bases.

Example : A weak acidic drug ibuprofen (pK_a = 4.4) is present in gastric juice (pH = 1.4) separated from plasma (pH = 7.4) by lipoid membrane of the gastric mucosa. The concentration ratio of unionised to ionised forms, total drug concentration ratio, and per cent of ionised and ionised forms in the gastric juice and plasma at equilibrium can be calculated from the Henderson-Hasselbalch equation as mentioned below. Further, the influence of pH of the media and pK_a of the drug on the process of diffusion may also be easily interpreted.

a. Relative amounts of unionised and ionised forms:
For weak acids, the Henderson-Hasselbalch equation is

$$pK_a - pH = \log \frac{\text{Concentration of unionised drug [U]}}{\text{Concentration of ionised drug [I]}}$$

Gastric juice (pH = 1.4)

$$pK_a - pH = \log \frac{[U]}{[I]}$$

$$4.4 - 1.4 = \log \frac{[U]}{[I]}$$

$$3 = \log \frac{[U]}{[I]}$$

Plasma (pH = 7.4)

$$pK_a - pH = \log \frac{[U]}{[I]}$$

$$4.4 - 7.4 = \log \frac{[U]}{[I]}$$

$$-3 = \log \frac{[U]}{[I]}$$

Taking antilog of both sides

$$1000 = \log \frac{[U]}{[I]}$$

If [U] = 1, then [I] = 0.001

$$0.001 = \log \frac{[U]}{[I]}$$

If [U] = 1, then [I] = 1000

Thus at equilibrium:

(*i*) The concentration ratio of unionised to ionised drug in gastric juice is 1: 0.001, and in plasma is 1: 1000

(*ii*) The total drug concentration (unionised and ionised) in gastric juice is 1 + 0.001 = 1.001, and in plasma is 1 + 1000 = 1001.

The example is also depicted in Figure 4.3.

b. The total drug concentration ratio between plasma and gastric juice is

$$\frac{\text{Total plasma concentration}}{\text{Total gastric juice concentration}} = \frac{1001}{1.001} = 1000$$

c. Per cent of ionised and unionised forms

The percent-ionised form of an acid drug can be calculated form the following formula:

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}\,(pK_a - pH)}$$

Gastric juice (pH = 1.4)

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}\ (4.4 - 1.4)}$$

$$= \frac{100}{1 + \text{antilog}\ (3)}$$

$$= \frac{100}{1 + 1000}$$

$$= \frac{100}{1001}$$

$$= 0.099 = 0.1$$

Therefore:

Ionised form = 0.1%

Unionised form = 99.9% (100 – 0.1)

Plasma (pH = 7.4)

$$\% \text{ Ionised} = \frac{100}{1 + \text{antilog}\ (4.4 - 7.4)}$$

$$= \frac{100}{1 + \text{antilog}\ (-3)}$$

$$= \frac{100}{1 + 0.001}$$

$$= \frac{100}{1.001}$$

$$= 99.9$$

Ionised form = 99.9%

Unionised form = 0.1% (100 – 99.9)

It is apparent from the above example that degree of ionisation of an electrolyte drug depends on its pK_a value and the pH of the environment. As the pK_a value for a drug is constant (pK_a of a compound is a measure of its inherent acidity or basicity, and is determined by the molecular arrangement of the constituent atoms), changes in pH of the environment primarily determines the degree of ionisation. For a weak acidic drug, when the pH is lower than the pK_a, the un-ionized form predominates and when the pH is higher than pk_a, the ionized form predominates. Accordingly for ibuprofen (pK_a 4.4), the ratio of un-ionized to ionized form in gastric fluid (pH 1.4) is 1000:1; in plasma (pH 7.4) the ratio is reversed (1:1000).

As a general rule, when pH of medium and pK_a of drug are equal, 50% of drug will be in ionised and 50% in unionised forms. When the pH is one unit below the pK_a, an acid is 9% ionised (91% unionised) and a base is 91% ionised (9% unionised). If the pH drops by 2 units from the pK_a, then acid becomes 1% ionised (99% unionised) and base becomes 99% ionised (1% unionised). The converse occurs when the pH shifts to the alkaline side i.e. if the pH is increased from the pK_a value by one unit, an acid becomes 91% ionised (9% unionised) and a base becomes 9% ionised (91% unionised), and so on. Broadly, each one pH unit change to the acid or alkaline side of the pK_a results in ten fold change in ionised form relative to unionised form (Table 4.2). Therefore, when a weak acid is given orally, most of the drug in the stomach is un-ionized, favoring diffusion through the gastric mucosa. For a weak base with a pK_a of 4.4, the outcome is reversed; most of the drug in the stomach is ionized. Theoretically, weakly acidic drugs (e.g. aspirin) are more readily absorbed from an acid medium (stomach) than are weakly basic drugs (e.g. quinidine).

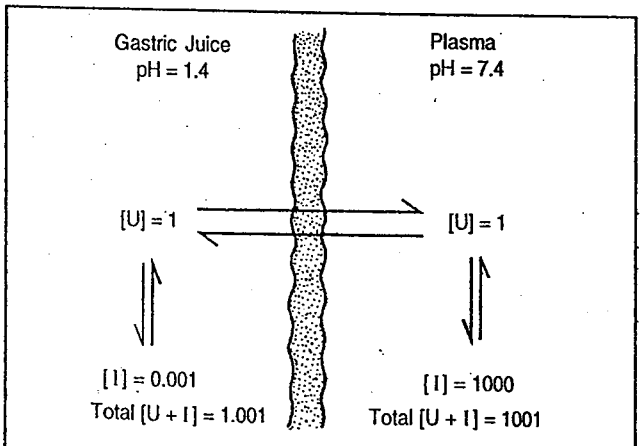


Fig. 4.3. Effect of pH on the distribution of a weak acid (pH 4.4) between gastric juice (pH=1.4) and blood plasma (pH = 7.4) (See text for detail).
Abbreviations : U = unionised; I = ionised

Table 4.2: Influence of pH on ionisation of an acidic drug ibuprofen (pK_a 4.4) and a basic drug quinidine (pK_a 4.4).

pH	Ibuprofen (Acidic drug) Per cent		Quinidine (Basic drug) Per cent	
	I	U	I	U
4.4	50	50	50	50
3.4	9	91	91	9
2.4	1	99	99	1
1.4	0.1	99.9	99.9	0.1
5.4	91	9	9	91
6.4	99	1	1	99
7.4	99.9	0.1	0.1	99.9

Abbreviation: I = ionised; U = unionised.

Influence of pH and pK_a on diffusion: From the example given above and Figure 4.3, it may be concluded that:

(*i*) The total drug concentration (unionised + ionised) ratio between plasma and gastric juice at equilibrium is 1000 : 1. This indicates that even by simple diffusion, high amounts of drugs can be transferred from one body compartment to another.

(*ii*) The total amount of drug (unionised + ionised) is more on that side of membrane (plasma) where the drug exists in the ionised form. This is mainly because the drug in ionised form is insoluble in lipid and, therefore, is non-diffusible. This phenomenon is called ion-trapping i.e. ions remain trapped on one side of the biological membrane.

(*iii*) The concentration of unionised drug is same on both sides of the membrane. This is because the unionised form is diffusible and achieves equilibrium. [Static equilibrium is practically not achieved in many instances, particularly when the drug diffuses from a smaller volume into a much larger volume (e.g. blood), or when the drug is rapidly removed by metabolism and/or excretion. In such cases, the diffusion process may remain unidirectional].

(*iv*) The degree of dissociation of the drug on each side depends on the pH of the plasma and gastric juice. The total concentration difference between the two sides, therefore, is a direct function of the pH gradient across the membrane.

2. Filtration

Filtration (Pore filtration) is the simple passage of drugs through pores or channels in the membrane. The process is important in the transfer of many small (Mol. wt. < 100), polar and non-polar substances. The driving force for filtration is constituted by the hydrostatic pressure or the osmotic differences across the membrane due to which water flows through the aqueous pores. This passive (energy independent) bulk fluid movement carries or drags solute molecules through the pores in the moving stream,

provided that the soluble molecules are smaller than the diameter of pores or aqueous channels.

Permeation through water-filled channels is of lesser importance for most drugs because majority of cell membranes (e.g. intestinal mucosa, tissue cells and erythrocytes) have very small pore sizes (4Å). However, capillary endothelial cells (except those in brain) have large pores (40Å) and many large molecules can filter through them (capillary membrane is composed of an interlocking mosaic of endothelial cells separated by slits which could serve as pores). For most tissues, filtration through pores or channels occurs only for molecules less than 150-200 Mol. wt. In the endothelial capillary lining, the relatively large pores allow substances of molecular weight of 20-30,000 to pass. Filtration is of particular importance in renal excretion (glomerular filtration) of drugs, removal of drugs from cerebrospinal fluid, and passage of drugs across hepatic sinusoidal membrane.

II. Specialised Transport

1. Active Transport

Active transport is an energy dependent transport system in which the solute takes help of a carrier to cross the barrier imposed by lipid bilayer. The carrier is usually a membrane protein (e.g. enzyme) that binds reversibly with the solute molecule on exterior membrane surface to form the carrier-solute complex. This carrier-solute complex then traverses across the membrane to the other side where it dissociates and discharges the solute molecule (Figure 4.4). The carrier then returns to its original site to repeat the cycle by accepting a fresh molecule of solute. The movement of solute occurs against the concentration or electrochemical gradient (from lower to higher concentration; up-hill movement), therefore, the process requires expenditure of energy. After transportation of the solute, energy is also required to energise the carrier for repeating the cycle.

Unlike simple diffusion and filtration, the active transport is a structure specific process i.e. the carriers possess special affinity for and transport drugs of specific chemical structures only. Generally, these carriers have special affinity for essential nutrients like sodium, potassium, glucose, and certain amino acids and vitamins. Because the number of carriers is limited in biological membranes, the process often becomes saturated, particularly at high concentrations of drugs due to non-availability of free carriers. As the carriers are also structure specific, the transport process is subject to competition between agents having similar structures. The active transport is not very common in body because it occurs only from selected sites, which are rich in number of carriers.

Active transport is generally considered important for large hydrophilic, polar or electrolyte substances. Drugs having structural similarities to endogenous substances are primarily transported actively via the carrier. Examples include L-dopa, methyldopa, some antimetabolites (methotrexate, 5-bromouracil and 5-fluorouracil) and lithium. Active transport is also important in renal and biliary excretion of many drugs and their metabolites, and in removal of certain drugs (e.g. penicillins) from the CNS. Drugs those are analogues of such molecules compete with these substrates and are transported.

Types of active transport

Active transport can be further divided into two subtypes: primary and secondary active transports. In primary active transport, only one substance is taken by the carrier and the energy of ATP hydrolysis is directly applied to the carrier protein for the transport of substrate against its concentration or electrochemical gradient. In secondary active transport, two solutes share a common carrier, out of which one is driving solute (usually a cation like Na^+, K^+ or Ca^{++}) and other is a substrate that is transported against the concentration gradient. Cell energy is utilised here to create an electrochemical gradient for the driving solute, and the free energy of driving solute is then utilised to transport the substrate. When the substrate moves in the same direction of the driving solute, the process is called co-transport or symport (e.g. sodium co-transport of glucose and amino acids in the intestinal epithelial cell), and when the substrate moves in the opposite direction of the driving solute it is called counter-transport or antiport (e.g. sodium counter transport of hydrogen ion). Secondary active transport is important for body ions and nutrients and is of minor importance for drugs (see also Chapter 38).

Table 4.3: Comparative features of simple diffusion and active transport process.

Features	Simple diffusion	Active transport
1. Energy	Energy independent (Passive process)	Energy dependent (Active process)
2. Carrier	Not required	Required
3. Movement of drug	Along concentration gradient (down-hill movement)	Against concentration gradient (up-hill movement)
4. Saturation	Non-saturable process	Saturable process
5. Structure specificity	Not required	Required
6. Competitive inhibition	Does no occur	May occur
7. Nature and type of drug	Lipid soluble and unionised	Mainly hydrophilic, polar and electrolyte
8. Occurrence	Wide occurrence in body	Limited to certain sites

2. Facilitated Diffusion

Facilitated diffusion is a carrier mediated transport system that operates along the concentration gradient (down-hill movement). Although in this type of transport, a carrier facilitates movement of drugs across the membrane, the driving force is the concentration gradient. Hence, it does not require energy and is a passive process. The facilitated transport proceeds more rapidly than the simple diffusion, and can translocate certain non-diffusible substances. Similar to active transport, it is structure specific and saturable process and is subject to competition between agents having similar structures. However, unlike active transport which mainly uses symports and antiports, facilitated diffusion utilises uniports (Carrier proteins which actively or passively transport only one molecule through the membrane). Examples of facilitated transport system include entry of glucose into erythrocytes, intestinal absorption of vitamins B_1 and B_2, and vitamin B_{12} along with intrinsic factor. Facilitated transport is not a major process for drug transport.

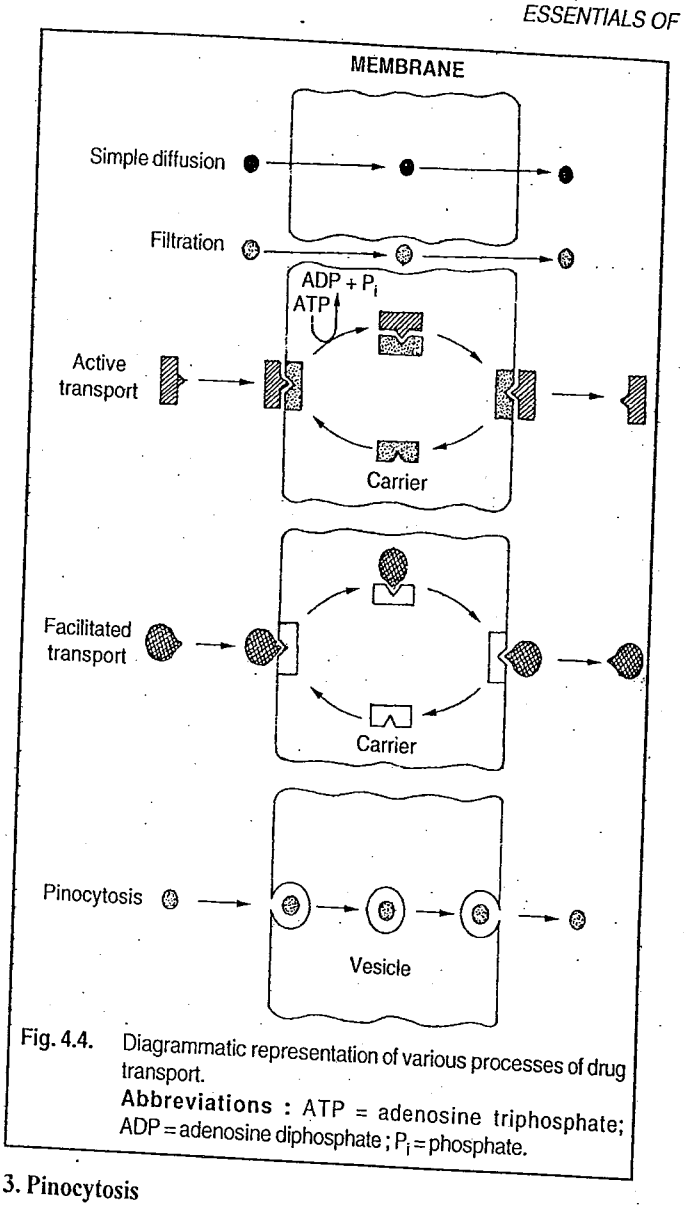


Fig. 4.4. Diagrammatic representation of various processes of drug transport.
Abbreviations : ATP = adenosine triphosphate; ADP = adenosine diphosphate ; P_i = phosphate.

3. Pinocytosis

Pinocytosis is a minor transport mechanism, which involves engulfing extra cellular drug droplets within a segment of the cell membrane to form saccules or vesicles. These vesicles are then pinched-off intracellularly (interiorised or internalised) and transported across cell membranes where they release the engulfed substance. Pinocytosis is similar to phagocytosis that involves the invagination of the membrane to enclose a solid particle. Both pinocytosis and phagocytosis are sometimes taken together as endocytosis. Pinocytosis, like active transport, requires the expenditure of cellular energy and shows structure selectivity (although of low order), and competitive and saturation kinetics. Pinocytosis is responsible for the cellular uptake of macromolecular nutrients like fats, starch, proteins, oil soluble vitamins (e.g. A, D, E and K), and some drugs such as insulin. Orally administered Sabin Polio vaccine and large protein molecules are thought to be absorbed by pinocytosis. Insoluble particles such as those of titanium dioxide and asbestos are known to be absorbed by phagocytosis in the lungs.

ABSORPTION

Absorption is defined as the process of movement of unchanged drug from its site of administration to the blood stream.

Except for the intravascular routes (intravenous and/or intra-arterial), absorption is a prerequisite for all systemic routes because drugs can exert their pharmacological actions only when they come into blood circulation from their site of administration. During this process, drugs have to cross the biological membranes. Both the rate and the extent of drug absorption are important because they determine the duration and intensity of drug action. A drug that is completely but slowly absorbed may fail to show therapeutic response as the minimum effective concentration is never reached. On the other hand, a rapidly and completely absorbed drug attains the therapeutic level easily to elicit its pharmacological effect. Regardless of the route of administration, drugs must be in solution form to be absorbed. Thus, solid forms (e.g. powders, capsules and tablets) must be able to disintegrate and dissolve in body fluids.

Factors Affecting Absorption of Drugs

The rate and extent of absorption are affected by following important factors:

a. ***Physico-chemical properties of drug:*** Important physico-chemical properties which influence drug absorption are lipid solubility (oil: water partition coefficient), pK_a and molecular size of the drug. As mentioned earlier, lipid soluble and unionised drugs are rapidly and efficiently absorbed into the blood circulation.

b. ***Nature and type of dosage form:*** Nature and type of dosage form affects absorption to a greater extent. Drugs given in aqueous solutions are more rapidly absorbed than those given in oily, suspension or solid form because these mix more readily with the aqueous phase at the absorptive site. For drugs given in solid form, rate of dissolution (amount of solid substance that goes into solution per unit of time) also determines absorption. As a general rule, the absorption of a drug from various dosage forms occurs in the following order: solutions > emulsions > suspensions > capsules > tablets > sustained release products.

c. ***Concentration and volume:*** Drugs given in high concentration/dose or volume are absorbed more rapidly than those given in dilute concentration and small volume. This is because high concentration and large volume develop high concentration gradient, an essential requirement for passive diffusion.

d. ***Blood flow to site of administration:*** Better perfusion rate of the absorptive tissue enhances absorption of the drug. This is because blood circulation removes the drug from the site of absorption and maintains concentration gradient across the membrane.

e. ***Area of absorbing surface:*** Drugs are absorbed more rapidly from the site having a large surface area than that possessing small area. For example, drugs are more rapidly absorbed from the intestine than from the stomach.

f. ***Route of administration:*** Absorption of drugs from IM sites is relatively rapid, but much slower in comparison to IP injections. Absorption of drugs from SC sites is slower than those from IM sites, but is faster than the oral route. Absorption of lipid soluble drugs from pulmonary mucosa is very rapid because of its rich vascular and vast surface area.

g. *Disease states:* Several disease states can influence the rate and extent of drug absorption. For example, acid-base imbalance may alter body fluid pH, infection may alter membrane permeability, and cardiovascular disease may affect blood flow to absorbing sites.

Gastrointestinal Tract

The oral route of drug administration is commonly adopted for systemically acting drugs. Drug administered by oral route finds its way quickly to the stomach, passing through the mouth and oesophagus. The oral mucosa has a thin epithelium and rich vascularity, which favor absorption; however, contact time in mouth is usually very brief for substantial absorption to take place. Drug absorption also does not normally occur in the oesophagus due to rapid transmit time. The effective barrier to orally administered drugs is the epithelial lining of gastro-intestinal tract. The ways in which drugs cross GI mucous membranes are governed by the fundamental principles of drug transport as discussed earlier. Lipid soluble and unionised drugs are readily absorbed by diffusion from stomach as well as intestine at rates proportional to their lipid: water coefficient. According to the pH-partition hypothesis, acidic drugs are best absorbed from stomach (acidic pH), and basic drugs from intestine (alkaline pH) in which conditions they are unionised to a large extent. However even for acidic drugs, absorption from stomach is less because the gastric mucosa is thick and covered with mucus, and the surface area is small. Therefore, practically most acidic and basic drugs are absorbed mainly from the intestine (intestine has large surface area, high perfusion rate, long residence time of drug, and pH range of 5 to 7.5). Some drugs are small enough to undergo filtration through the pores in the membranes. Uptake of sugar and other nutrients occur by active transport system.

Presence of food in the GI tract often dilutes the drug and retards its absorption. Gastrointestinal fluids usually promote disintegration (breakdown of solid dosage forms into small particle) and dissolution (solid to liquid phase) of dosage forms, but may inactivate some drugs (e.g. penicillin-G by gastric acid and insulin by peptidases). Drug biotransformation within the ruminal and intestinal lumen by microflora or within the mucosa by host enzyme systems also retards drug absorption and makes certain drugs ineffective orally (e.g. catecholamines). Generally, rapid gastric emptying (passage from stomach to small intestine) increases the drug absorption (because most drugs are absorbed from small intestine), but rapid intestinal motility decreases the drug absorption (because drugs are rapidly passed through intestine). Absorption of drugs can also be greatly affected by other concurrently ingested drugs (See for detail drug interactions, Chapter 6). Several other confounding factors may affect the absorption of drugs from the GI tract and may result in erratic responses. These include molecular size and shape of drug, type of dosage form, morphological and functional differences of GI tract among various animal species, volume and osmolality of gastro-intestinal fluid and contents, intestinal blood and lymph flow, and pathological condition of gastric and intestinal epithelium.

Parenteral Sites

Drug absorption from intramuscular and subcutaneous sites is rapid as drugs are deposited directly in vicinity of the capillaries. Lipid soluble drugs pass readily across the whole surface of the capillary endothelium. Capillaries being highly porous do not obstruct absorption of even large lipid insoluble or ionised drugs. Substances with molecular weights >20,000 Da are mainly taken up into lymphatics. Thus, many drugs which are not absorbed orally are able to reach systemic circulation when given parenterally.

Absorption of drugs from IM sites is relatively rapid but depends on the vascularity of the injection site. Aqueous solutions of drugs are usually absorbed from the IM injection site within 10-30 minutes, provided blood flow is unimpaired. Absorption of drugs from a SC site is generally slower than that from IM site due to less perfusion, but this factor is useful for drugs for which a rapid response is not required (e.g. depot preparations). Incorporation of hyaluronidase facilitates drug absorption from SC injection by promoting spread, while addition of vasoconstrictors (e.g. epinephrine) retards drug absorption. Increasing blood supply to the IM or SC injection site by application of heat, massage or exercise hastens rate of absorption, while immobilisation of site, local cooling, or application of a tourniquet retards drug absorption.

Pulmonary Sites (Alveoli)

Some drugs intended for systemic effects are administered by inhalation either as gases (e.g. volatile and gaseous anaesthetics) or aerosols. The volatile and gaseous anaesthetics are absorbed practically instantaneously because they have relatively high lipid to water coefficients and generally are small molecules. The large surface area of alveoli, high permeability of alveolar epithelium and rich perfusion rate also facilitate their rapid absorption. In case of aerosols, the drug delivery to lungs is largely dependent of the particle size of the aerosolised droplets. Larger particles remain confined to the upper respiratory tract and do not reach the pulmonary tree, whereas small particles reach deep into the respiratory tract and may cause rapid absorption from alveoli (See for detail Routes of Drug Administration, Chapter 3).

Topical Sites

Topical application of drugs to skin or mucous membranes is mainly meant for local effects. However, drugs may be absorbed through the skin or mucous membranes, or in some cases are intended to be absorbed to produce systemic effects. Systemic absorption after topical application depends primarily on lipid solubility of the drugs; lipid insoluble drugs generally penetrate the skin or mucous membranes poorly. Absorption of drugs through skin is usually poor because the keratinised epidermis behaves like a barrier. However in some cases, it may be enhanced by rubbing, use of occlusive dressing or rarely by iontophoresis, if compound is ionised. Certain solvents (e.g. dimethyl sulphoxide; DMSO) may facilitate the penetration of drugs through skin. Inflammation, cuts, rashes, mild burns, etc, promote drug absorption through skin because these conditions destroy the epidermis. In recent times, several topical formulation of drugs have come where topical application is intended for systemic actions.

Absorption of most drugs through mucous membranes is usually more rapid than the skin because mucous membranes are thin and highly vascular. Systemic absorption through mucous membranes often results in unwanted side effects. However, some drugs are applied to the mucous membranes to facilitate absorption and produce systemic effects.

DISTRIBUTION

Distribution may be defined as a process by which drugs reversibly leave the blood stream and enter the extravascular fluid and tissues. Distribution is an important pharmacokinetic process as it determines the transport of drugs to their site of action and to the organs of metabolism and excretion. Distribution of a drug is not uniform throughout the body because of difference in perfusion rates. The extent to which a drug is distributed to a particular tissue or organ depends largely on its ability to penetrate the capillary endothelium and cross the cell membrane. The tissue permeability of a drug is determined by a number of factors including the physico-chemical properties of the drug, binding to plasma and tissue proteins, blood flow, special compartments and barriers, disease states, etc. Some important factors which govern distribution of drugs are considered briefly below.

1. Physicochemical Properties of the Drug

Distribution of drugs to different body tissues is greatly influenced by physico-chemical characteristics of the compound. Almost all drugs having molecular weight less than 500 to 600 Da easily cross the capillary membrane to penetrate into the extracellular fluids (except in CNS) because junctions between the capillary endothelial cells are not tight. However, passage of drugs from the ECF into the cells is a function of molecular size, degree of ionisation and lipophilicity of the drug. As discussed earlier, a drug that is lipid soluble and unionised at blood and extracellular fluid pH permeates the cell more rapidly than a polar and ionised drug. Only small, water-soluble molecules and ions of size below 50 Da enter the cell through aqueous filled channels, whereas those of larger size are restricted unless a specialised transport system exists for them. According to the pH-partition hypothesis, basic drugs present in blood (pH 7.4) readily enter into acidic tissues and fluids, including the intracellular fluids (pH 7.0) and concentrate there (because basic drugs will be more unionised in plasma than that of acid fluid, so they will diffuse readily, but on entering the acid compartment they become ionised and get trapped). Conversely, acidic drugs attain high concentrations in the relatively more alkaline body fluids. For example, weak organic bases administered parenterally diffuse passively from blood (pH 7.4) into rumen fluid (pH 5.5 –6.5) of cattle and sheep, where they become trapped by ionisation. Similarly, weak bases tend to accumulate in milk since the pH of milk is slightly acidic (pH 6.5 to 6.8) to the blood.

2. Binding to Plasma and Tissue Proteins

Binding to plasma proteins

Drugs are transported in the circulating blood in two forms: free form and bound to plasma proteins. Free form of drugs is mostly dissolved in plasma water and is pharmacologically active, diffusible and available for metabolism and excretion. Protein bound drugs are pharmacologically inactive, non-diffusible, and not available for metabolism or excretion as they cannot pass through capillaries and cell membranes because of their larger size. Plasma protein binding is an important phenomenon, which significantly affects distribution and pharmacological and therapeutic response of drugs.

Plasma protein binding of drugs generally involves weak chemical bonds and is a reversible process. Extent of binding is a characteristic of a drug and generally remains fixed for a drug within a wide range of concentration. The binding of individual drugs ranges from very little (e.g. aminoglycosides) to very high (e.g. warfarin) (Table 4.4). In circulating blood, there is a constant ratio between the bound and free fractions of the drug. When th concentration of the free drug falls due to redistribution, metabolism or excretion, the free: bound ratio is maintained by dissociation c the bound form. Thus plasma protein binding mainly serves as a reservoir, which supplies free drug whenever required.

Free drug ⟷ Protein bound drug

A large variety of drugs ranging from weak acids, neutra compounds and weak bases bind to plasma proteins. Commor blood proteins which drugs bind to are albumin, lipoprotein, glycoprotein and globulins. Acidic and neutral drugs generally bind to plasma albumin and basic drugs to α_1-acid glycoproteins; binding to other plasma proteins (e.g. lipoproteins and globulins occurs to a much smaller extent. For majority of drugs, binding to plasma albumin (Mol. Wt. 65,000), which comprises >50% o the total proteins, is quantitatively more important. The binding of drugs to albumin may show low capacity (one drug molecule per albumin molecule) or high capacity (two or more drug molecules per albumin molecule). The albumin can bind severa compounds having varied structures, some substances even to a single site. Groups of drugs those bind to the same site compete with each other for binding (see drug displacement interactions).

Some drugs may bind to blood components other than plasma proteins. Drugs like phenytoin, pentobarbital and phenothiazines bind to haemoglobin, and imipramine and chlorpromazine bind with the RBC membrane.

Table 4.4: Binding of some important drugs to plasma proteins in dogs.

Drug	Extent of binding (per cent)	Drug	Extent of binding (per cent)
Highly protein bound (>50%)		*Lowly protein bound (<50%)*	
Warfarin	99	Chloramphenicol	40
Phenylbutazone	98	Enrofloxacin	27
Propranolol	97	Digoxin	25
Digitoxin	89	Cefazolin	22
Valproic acid	80	Procainamide	15
Phenytoin	78	Amoxicillin	13
Quinidine	75	Morphine	12
Sulphisoxazole	68	Codeine	10
Ketamine	53	Theophylline	7

Significance of plasma-protein binding

a. Plasma protein bound drugs are non-diffusible. Therefore, high plasma protein binding reduces the efficiency of drug distribution.

b. Plasma protein bound drugs are pharmacologically inactive. Therefore, heavily protein-bound drugs are generally less efficacious and less potent.

c. Protein-bound drugs do not undergo metabolism and excretion through glomerular filtration. Therefore, heavily protein-bound drugs generally have long plasma half-lives.

d. Protein-bound drugs may show drug displacement interactions. Therefore, if a drug is heavily protein-bound, has a narrow therapeutic index and show small volume

of distribution, the displacement may significantly increase the biological activity and possible toxicity of the drug.

Drug displacement interactions: Drug displacement interactions occur between two or more drugs which bind to the same plasma protein site. If one drug is binding to such a site, then administration of second drug having higher affinity for the same site results in displacement of first drug from its binding site. In many cases, the impact of interactions is minimal (especially if the drug has large volume of distribution), but in some instances (heavily protein bound drug with limited volume of distribution), a slight displacement of first drug will result in marked increase in its biological activity. For example, administration of phenylbutazone to a patient on warfarin therapy results in displacement of warfarin from its binding site. Warfarin has high plasma protein binding of about 99% (free drug concentration ~1%), shows a small volume of distribution (remains confined to blood compartments) and has a narrow therapeutic index. If just 1% of warfarin is displaced by the phenylbutazone, the concentration of free warfarin will be doubled (~2%). This enhanced concentration of free warfarin may cause intense biological activity with severe haemorrhagic episodes, which may prove to be fatal. Kernicterus in human infants is another example of a disorder caused by displacement of bilirubin from albumin binding sites by non-steroidal anti-inflammatory drugs.

Binding to tissue proteins

A drug may bind to one or more of the several tissue components and may get accumulated there. A drug is said to be localised in tissue if its concentration in that tissue exceeds the concentration in plasma. Tissue-drug binding is important in distribution from two viewpoints: firstly it increases the apparent volume of distribution (in contrast to plasma protein binding which decreases it) and secondly it results in localisation of the drug at a specific site in the body. Binding of drugs with tissues may be either reversible where it acts as storage site, or irreversible. The irreversible binding usually occurs as a result of chemical activation of the drug, which then attaches strongly to the tissue protein by covalent chemical bonding. The reversible tissue binding is not harmful, but the strong irreversible chemical binding often results in adverse effects and toxicity. For example, irreversible covalent binding of paracetamol and chloroform metabolites with liver results in hepatotoxicity, deposition of tetracyclines in dental tissues during odontogenesis in infants and children results in permanent brown-yellow discolouration of teeth and interaction of sulphoxide of chlorpromazine with retina of Hounds breed of dogs leads to retinopathy. Some important examples of tissue-drug binding are given in Table 4.5.

Table 4.5: List of selected drugs having affinity for specific organ/tissue

Organ/tissue	Drug(s)
Liver	Chloroquine, paracetamol and epoxides of halogenated hydrocarbons.
Lungs	Imipramine, chlorpromazine and antihistamines.
Kidneys	Heavy metals (mercury, lead, cadmium) and digoxin.
Thyroid	Iodine.
Skin	Chloroquine and phenothiazines.
Eye (retina)	Chloroquine and sulphoxide of chlorpromazine.
Bone and Teeth	Tetracyclines, fluoride and heavy metals (lead).
Adipose tissue	Thiopental, minocycline and DDT.
Hair	Arsenicals.

3. Blood Flow and Organ Size

The rate of blood flow to tissue capillaries varies widely as a result of unequal distribution of cardiac output to various organs. In addition to the physico-chemical properties of drug and plasma protein binding, the drug distribution to a particular organ or tissue depends on the size of tissue (tissue volume) and tissue perfusion rate (volume of blood that flows per unit time per unit volume of tissue). Highly perfused tissues such as lungs, kidneys, liver, heart, adrenals and brain are rapidly equilibrated with lipid soluble drugs. Muscle and skin are moderately perfused, so they equilibrate slowly with the drug present in blood. Adipose tissues, bones and teeth being poorly perfused take longer to get distributed with the same drug.

4. Specialised Compartments and Barriers

Some organs/areas of the body are not readily accessible to drugs because their anatomic make-up poses permeability restriction to drugs. Some of these compartments and barriers include blood-brain barrier, blood-CSF barrier and placental barrier.

Blood-brain barrier: The capillary endothelial cells in brain have tight junctions and lack pores or gaps. Surrounding the tight and overlapping endothelial layer is a continuous basement membrane. The basement membrane in turn is enveloped by a 'perivascular foot process' formed by astrocyte cells which encircle about 85% of the surface areas of brain capillaries (Figure 4.5). Together these layers add up to a formidable non-polar barrier called the blood-brain barrier (BBB). Since the BBB is a lipoidal barrier, it allows only the drugs having high to moderate lipid solubility (oil/water partition coefficient) to diffuse passively; ionised or polar drugs largely fail to enter the CNS. Some water soluble drugs (e.g. l-dopa and methyl dopa) and endogenous substances (e.g. sugars and amino acids) are transported to brain by carrier mediated transport system. There are certain specific sites in brain like chemoreceptor trigger zone (CTZ) and posterior lobe of hypothalamus where the BBB does not exist, and at these sites brain may be exposed to some lipid insoluble and polar drugs also. In newly born and old animals, the blood-brain barrier is generally less effective, allowing increased passage of water soluble and polar compounds into the brain.

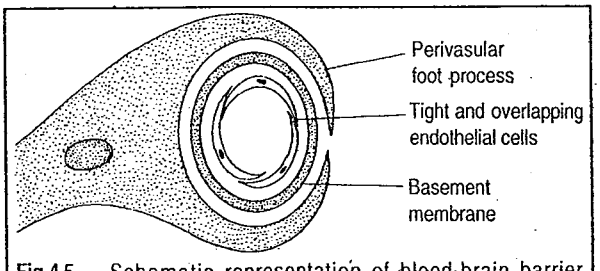


Fig.4.5. Schematic representation of blood-brain barrier (see text for detail).

Blood-cerebrospinal fluid barrier: The blood-cerebrospinal fluid (CSF) barrier is formed mainly by the choroid plexus of lateral, third and forth ventricles. Unlike capillary endothelial cells in brain, the capillary endothelium that lines the choroid plexus have open junctions or gaps but the choroidal cells are joined to each other by tight junctions. Therefore for a drug to enter CSF from the blood, it can move within the open junctions between capillary endothelial cells but must pass through the tightly linked choroidal epithelial cells forming the blood-CSF barrier. Overall, the blood-CSF barrier has permeability characteristics similar to those of BBB (i.e. allows only lipid soluble drugs to enter the CSF), but unlike brain a drug that enters the CSF slowly generally fails to achieve high concentration there because the bulk flow of CSF continuously removes the drug. Therefore for a given drug, its concentration in brain is always higher than that in the CSF.

Placental barrier: The maternal and foetal blood vessels are separated by a layer of trophoblastic cells which together constitute the placental barrier. The characteristics of this lipid barrier are generally the same as of blood-brain barrier. Lipid soluble drugs pass freely into foetal circulation by diffusion and water-soluble drugs or essential nutrients cross the placental barrier by carrier mediated transport system. Restricted amounts of lipid insoluble drugs, especially when present in high concentration or for long periods in maternal blood, also gain access to the foetus by non-carrier mediated processes. Therefore, the placental barrier is not as effective as the blood-brain barrier and impermeability of the placental barrier to polar compounds is relative rather than absolute. So care must be taken while administration of all types of drugs during pregnancy because of the uncertainty of their harmful effects on developing foetus.

Other barriers: The prostrate, testicles and globe of eyes contain barriers which prevent easy drug penetration to tissues. Drugs also have relatively restricted access to pericardial fluid, bronchial secretions and fluid in the middle ear. Lipid soluble drugs can penetrate and reach these structures freely, whereas water-soluble drugs entry is restricted.

5. Availability of Specialised Transport System

Some drugs are concentrated in some specific tissues as a result of their active uptake by specialised transport systems present in such tissues. For example, iodine is actively concentrated into the thyroid cells and guanethidine is distributed into the adrenergic nerve terminals.

6. Disease States

Distribution characteristics of several drugs are altered in disease states. In meningitis and encephalitis, the blood-brain barrier becomes more permeable and the polar antibiotics like penicillin-G, which do not normally cross it, gain access to the brain. In hypoalbuminaemia, plasma protein binding of drugs may be reduced and high concentration of free drugs may be attained. In congestive heart failure or shock, the perfusion rate to the entire body decreases that adversely affects distribution of drugs.

7. Physiological Factors

Several physiological factors also contribute significantly to the distribution of drugs. Marked differences in the distribution pattern of a drug in different age groups have been noted. In infants, the blood-brain barrier is poorly developed, myelin content is low and cerebral blood flow is more, all of which provi greater penetration of drugs in the brain. In geriatric animals, t protein contents are low and blood-brain barrier is not efficie which alter distribution pattern of certain drugs. In obese anima the high adipose tissue can take up a large fraction of lipophi drugs despite the fact that perfusion rate to fats is low. The f content is also higher and albumin content is lower in bo infants and older animals. During pregnancy, the growth of uteru placenta and foetus increases the volume available for distributio of drugs. The foetus also represents a separate compartment which a drug can distribute.

METABOLISM/BIOTRANSFORMATION

Metabolism or biotransformation may be defined as th conversion from one chemical form of a substance to another There is a technical difference between the terms metabolism an biotransformation (see the definitions given below), but bot terms are used interchangeably in pharmacology and carry the same meaning (in practice, the term metabolism is commonly use probably because products of drug transformation are called metabolites). Metabolism is an essential pharmacokinetic proces that renders the lipid soluble and non-polar compounds to the water soluble and polar compounds so that they are easily excrete by various processes. This is because only water-soluble and polar substances undergo excretion, whereas lipid soluble an non-polar substances are passively reabsorbed from renal or extra-renal excretory sites by virtue of their lipophilicity. It has been observed that if a lipid soluble substance is not converted into a water-soluble form, it may remain in body for very long period (weeks, months or years) and precipitate toxic reaction. Thus metabolism is a necessary biological process that limits the pharmacological action and life of a drug in the body.

Biotransformation: It is a specific term used for chemical transformation of xenobiotics in the body/living organism.

Metabolism: It is a general term used for chemical transformation of xenobiotics and endogenous nutrients (e.g. proteins, carbohydrates and fats) within or outside the body.

Functions of Metabolism

Metabolism performs one or more of the following functions in body:

a. It causes conversion of an active drug to inactive or less active metabolite(s) called inactivation or pharmacological inactivation e.g. phenobarbital, amfetamine, morphine, phenytoin and propranolol.

b. It causes conversion of an active drug to more active metabolite(s) called bioactivation or toxicological activation e.g. codeine, isoniazid and malathion.

c. It causes conversion of an inactive drug (pro-drug) to active metabolite(s) called pharmacological activation e.g. enalapril, phenacetin, pivampicillin and levodopa.

d. It causes conversion of an active drug to equally active metabolite(s) (no change in pharmacological activity) e.g. digitoxin, diazepam, amitriptyline and phenylbutazone.

e. It causes conversion of an active drug to active metabolite(s) having entirely different pharmacological activity (change in pharmacological activity) e.g. iproniazid.

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Table 4.6: Functions of metabolism.

Drugs	Metabolites
Pharmacological Inactivation	
Phenobarbital	p-Hydroxyphenobarbital
Phenytoin	p-Hydroxyphenytoin
Procaine	p-Aminobenzoic acid
Griseofulvin	6-Demethylgriseofulvin
Bioactivation/Toxicological activation	
Codeine	Morphine
Paracetamol	Imidoquinone of N-hydroxylate metabolite
Sulphonamides	Acetyl derivatives
Malathion	Malaoxon
Halothane	Trifluoroacetic acid
Pharmacological activation	
Phenacetin	Paracetamol
Enalapril	Enalaprilat
Pivampicillin	Ampicillin
Sulphasalazine	5-Aminosalicylic acid
Levodopa	Dopamine
No change in pharmacological activity	
Digitoxin	Digoxin
Diazepam	Nordiazepam
Amitriptyline	Nortriptyline
Phenylbutazone	Oxyphenbutazone
Change in pharmacological activity	
Iproniazid (antidepressant)	Isoniazid (antitubercular)

Sites of Metabolism

Liver is the primary site for metabolism of almost all drugs because it is relatively rich in a large variety of metabolising enzymes. However, all tissues in body carry some degree of metabolic activities. Metabolism by organs other than liver (called extra-hepatic metabolism) is of lesser importance because lower level of metabolising enzymes is present in such tissues. Within a given cell, most drug metabolising activity is found in the smooth endoplasmic reticulum and cytosol. Drug metabolism can also occur in mitochondria, nuclear envelope and plasma membrane. A few drugs are also metabolised by non-enzymatic means called non-enzymatic metabolism. For example, atracurium, a neuromuscular blocking drug, is inactivated in plasma by spontaneous non-enzymatic degradation (Hoffman elimination) in addition to that by pseudocholinesterase enzyme.

Drug Metabolising Enzymes

A large variety of enzymes in animals are capable of metabolising drugs. These enzymes are located mainly in the liver, but may also be present in other organs like lungs, kidneys, intestine, brain, plasma, etc. Majority of drugs are acted upon by relatively non-specific enzymes, whose activities are directed more towards the types of molecule rather than the specific drugs. The drug metabolising enzymes can be broadly divided into two classes: microsomal and non-microsomal enzymes.

Microsomal enzymes: The microsomal enzymes are a variety of enzymes located within the lipophilic membranes of the smooth endoplasmic reticulum (SER) of liver and other tissues. The endoplasmic reticular enzymes are called microsomal enzymes because when the SER is isolated in laboratory by tissue homogenation and differential centrifugation, the cells are disrupted and the SER membrane re-forms into minute spherical vesicles called microsomes; all enzymes present in vesicles thus becomes microsomal enzymes. Microsomal enzymes are the most important group of enzymes which catalyze glucuronide conjugation, most oxidative reactions, and some reductive and hydrolytic reactions. The monooxygenases and glucuronyl transferase are important microsomal enzymes. Microsomal enzymes are inducible by drugs, diets and other factors (see induction of microsomal enzymes).

Non-microsomal enzymes: Enzymes occurring in organelles/sites other than endoplasmic reticulum (microsomes) are called non-microsomal enzymes. These are mostly present in the cytoplasm, mitochondria, body fluids etc. and occur mainly in the liver, GI tract, plasma and other tissues. They are usually non-specific enzymes which catalyse few oxidative reactions, some reductive and hydrolytic reactions and all conjugative reactions other than glucuronidation. None of the non-microsomal enzymes involved in drug biotransformation is known to be inducible.

PATHWAYS OF BIOTRANSFORMATION

Biotransformation of drugs has been divided into two major pathways or phases: Phase I and phase II.

Phase I: Phase I (Non-synthetic or non-conjugative phase) includes reactions which catalyse oxidation, reduction and hydrolysis of drugs. In phase I reactions, small polar functional groups like –OH, $–NH_2$, –SH, –COOH, etc. are either added or unmasked (if already present) on the otherwise lipid soluble drugs so that the resulting products may either undergo phase II reactions or are directly excreted. Phase I reactions generally precede phase II reactions.

Phase II: Phase II (Synthetic or conjugative phase) includes reactions those involve attachment of polar endogenous molecule such as glucuronic acid, sulphate, methyl, amino acids, etc., to either parent drugs or their phase I products. Phase II products, generally called conjugates, are generally water-soluble and polar metabolites those are readily excreted from the body. The products of phase II biotransformation may be further metabolized in body (sometimes termed phase III reactions) before final excretion.

Phase I reactions result in inactivation (mostly), no change in activity, or bioactivation, whereas phase II reactions generally result in inactivation (in some cases may cause bioactivation). Phase II reactions are also called synthetic reactions, in contrast to the non-synthetic phase I reactions, because the outcome of conjugative processes are generally products with increased molecular size and altered physico-chemical properties (it appears as if a new substance is synthesised). Some compounds pass through phase I reactions and then phase II reactions; others through one phase only (either) (Figure 4.6). Most drugs are metabolised by many reactions, simultaneously or sequentially, therefore a range of metabolites can be formed from a single drug simultaneously or one after the other in the body. The various phase I and phase II reactions are listed in Table 4.8.

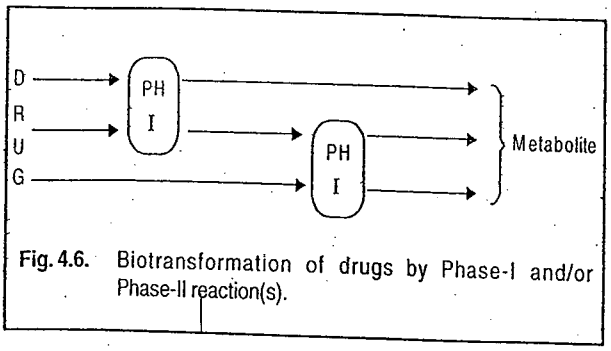


Fig. 4.6. Biotransformation of drugs by Phase-I and/or Phase-II reaction(s).

Phase I Reactions

1. Oxidation

Oxidative reactions are the most important metabolic reactions, as energy in animals is derived by oxidative combustion of organic molecules containing carbon and hydrogen atoms. The oxidative reactions are important for drugs because they enhance hydrophilicity of drugs by introducing polar functional groups such as –OH. Oxidation of drugs is non-specifically catalysed by a number of enzymes located primarily in the microsomes. Some of the oxidative reactions are also catalysed by non-microsomal enzymes (e.g. aldehyde dehydrogenase, xanthine oxidase and monoamine oxidase).

The most important group of oxidative enzymes are microsomal mixed function oxidases (MFO) or monooxygenases. These enzymes are located mainly in the hepatic endoplasmic reticulum (microsomes) and require both molecular oxygen (O_2) and reducing NADPH to effect the chemical reaction. The name mixed function oxidase was given to these enzymes in order to highlight the mixed function of the oxygen molecule, which is essentially required by them. The second term monooxygenases for the same enzymes was proposed as they incorporate only one atom of molecular oxygen into the organic substrate with concomitant reduction of the second oxygen atom to water. The overall stoichiometry of the reaction involving the substrate RH which yields the product ROH, is given by the following reaction:

$$RH + O_2 + NADPH + H^+ \xrightarrow{MFO} ROH + H_2O + NADP^+$$

The most important component of mixed function oxidases is the cytochrome P-450 (CYP-450), a microsomal superfamily of isoenzymes that catalyze the phase I oxidation of many xenobiotics. The wide distribution of cytochrome P-450 containing MFOs across all living creatures in varying organs makes it the most important group of enzymes involved in the drug biotransformation, accounting for about 75% of the total metabolism. CYP-450 enzymes can be induced or inhibited by many drugs and substances causing several drug interactions in which one drug either enhances the toxicity or reduces the therapeutic effect of another drug. With aging, the liver's capacity for metabolism through the CYP-450 enzyme system is reduced by more than 30% because liver volume and hepatic blood flow are decreased. In neonates the liver microsomal enzyme systems are not fully developed, so they also have difficulty in metabolizing many drugs. Accordingly, drugs those are metabolized by cytochrome P-450 system attain higher concentration and have prolonged half-lives in the neonates and older animals.

The cytochrome P-450 enzymes are superfamily of haem-thiolate proteins which bind to the substrate and activate oxygen. Cytochrome P-450 enzymes have been named on the basis of their cellular (*cyto*) location, spectrophotometric characteristics (*chrome*) and their absorbance of light wavelengths near 450 nm when the heme iron is reduced. These are predominantly present in liver but are also found in intestine, lungs, kidneys, brain etc. Depending upon the extent of amino acid sequence homology, the cytochrome P-450 (CYP) enzymes superfamily contains a number of isoenzymes, the relative amount of which differs among species and among individuals of the same species. These isoenzymes are grouped into various families designated by Arabic numbers 1, 2, 3, ..., (sequence those are greater than 40% identical belong to the same family), each having several subfamilies designated by capital letter A, B, C, ...(within the same family, sequence greater than 55% identical are in the same subfamily), while individual isoenzymes are again allotted Arabic numbers 1, 2, 3, ... (e.g. CYP1A1, CYP1A2, etc.). Each individual CYP isoform appears to have characteristic substrate specificity based on structural features of the substrate; considerable overlap, however, is present. In humans up to 18 families, 43 subfamilies and 57 genes of CYPs have been described. Out of these, CYP 1, 2 and

Table 4.7: Examples of some important drugs and the CYP450 isoforms catalysing their biotransformation

Drug	CYP450 Isoform	Drug	CYP450 Isoform
Alfentanil	3A4	Lidocaine	3A4
Amiodarone	3A4	Methadone	1A2, 2D6
Buspirone	3A4	Morphine	2D6
Caffeine	1A2	Naproxen	2C19, 2C9, 2A6
Carbamazepine	3A4	Nicotine	2A6, 2B6
Chlorpromazine	2D6, 3A4	Oestrogens	3A4
Cimetidine	3A4	Omeprazole	2C19, 2C8, 3A4
Codeine	2D6, 3A4	Paracetamol	1A2, 2E1, 3A4
Cyclophos-phamide	3B6, 3A4	Pethidine	2D6
Desipramine	1A2, 2D6	Propranolol	1A2, 2C19, 2D6
Dexamethasone	3A4	Quinidine	3A4
Diazepam	1A2, 2C19, 2C8, 3A4	Rifampicin	3A4
Enalapril	3A4	Selegiline	2D6
Erythromycin.	3A4	Tamoxifen	1A2, 2A6, 2B6, 2D6, 2E1, 3A4
Ethanol	2E1	Testosterone	3A4
Fentanyl	2D6, 3A4	Theophylline	1A2, 2E1, 3A4
Flecainide	2D6	Valproic acid	2C19
Halothane	2E1	Verapamil	3A4, 1A2, 2C9
Ibuprofen	2C9	Vincristine	3A4
Imipramine	1A2, 2C19, 2C9, 2D6, 3A4	Warfarin	2C19, 2C9
Ketoconazole	3A4	Yohimbine	2D6

3 account for 70% of total hepatic CYPs content and are responsible for more than 90% of drugs metabolism in liver; the isoenzymes CYP3A4 and CYP2D6 carry out biotransformation of largest number of drugs. Certain drugs (Table 4.7) are metabolised by more than one CYP isoenzymes. Cytochrome P-450s have also been extensively examined in mice, rats and dogs, in order to facilitate use of these model organisms in drug discovery and toxicology.

2. Reduction

Reduction is the acceptance of one or more electrons or their equivalent from another substrate. Reductive reactions, which usually involve addition of hydrogen to the drug molecule, occur less frequently than the oxidative reactions. Biotransformation by reduction is also capable of generating polar functional groups such as hydroxy and amino groups, which can undergo further biotransformation. Many reductive reactions are exact opposite of the oxidative reactions (reversible reactions) catalysed either by the same enzyme (true reversible reaction) or by different enzymes (apparent reversible reactions). Such reversible reactions usually lead to conversion of inactive metabolites into active drugs, thereby delaying drug removal from the body. Various reductive reactions with important examples are given in Table 4.8.

3. Hydrolysis

The hydrolytic reactions, contrary to oxidative or reductive reactions, do not involve change in the state of oxidation of substrate, but involve the cleavage of drug molecules by taking up a molecule of water. The hydrolytic enzymes which metabolise drugs are the same those act on endogenous substances and their activity is also not confined to the liver only but to many other organs including kidneys, intestine and plasma. A number of drugs with ester, ether, amide and hydrazide linkages undergo hydrolysis. Important examples are cholinesters, procaine, procainamide and pethidine.

Phase II Reactions

Phase II or Conjugation (Latin word *conjugatus* = yoked together) reactions involve combination of the drug or its phase I metabolite with an endogenous substance to form a highly polar product, which can be efficiently excreted from the body. In the biotransformation of drugs, such products or metabolites carry two parts: exocon, the portion derived from exogenous compound or xenobiotic, and endocon, the portion derived from endogenous substance. Conjugation reactions have high energy requirement and they often utilise suitable enzymes for the reactions.

The endogenous substances (endocons) for conjugation reactions are derived mainly from carbohydrates or amino acids and they generally possess large molecular size. They are also strongly polar or ionic in nature in order to render the substrate water-soluble. The molecular weight of the conjugate (metabolite) determines mainly its route of excretion. High molecular weight conjugates are excreted predominantly in bile (e.g. glutathione exclusively, glucuronide mainly), while low molecular weight conjugates are excreted mainly in the urine. As the availability of endogenous conjugating substance is limited, saturation of this process is possible and the unconjugated drug/metabolite may precipitate toxicity.

1. Conjugation with glucuronic acid/Glucuronidation

Conjugation with glucuronic acid (glucuronide conjugation or glucuronidation) is the most common and most important phase II reaction in vertebrates, except cats and fish. It is the only conjugation reaction that occurs in the liver microsomal enzyme system. Glucuronide conjugation makes most drugs more water soluble so that they are easily secreted in bile and readily eliminated in urine. The biochemical donor (cofactor) of glucuronic acid is uridine diphosphate α-D-glucuronic acid (UDPGA) and the reaction is carried out by enzyme uridine diphosphate-glucuronyl transferase (UDP-glucuronyl transferase; glucuronyl transferase). Glucuronyl transferase is present in microsomes of most tissues but liver is the most active site of glucuronide synthesis. Glucuronidation can take place in most body tissues because the glucuronic acid donor UDPGA is present in abundant quantity in body, unlike donors involved in other phase II reactions. In cats, there is reduced glucuronyl transferase activity, while in fish there is deficiency of endogenous glucuronic acid donor. The limited capacity of this metabolic pathway in cats may increase the duration of action, pharmacological response and potential of toxicity of several lipid-soluble drugs (e.g. aspirin) in this species. A large number of drugs undergo glucuronidation including morphine, paracetamol and desipramine. Certain endogenous substances such as steroids, bilirubin, catechols and thyroxine also form glucuronides. Aging does not affect glucuronidation, but in neonates the process of glucuronide conjugation is much slower, which sometimes may produce serious effects.

Deconjugation process: Occasionally some glucuronide conjugates those are excreted in bile undergo deconjugation process in the intestine mainly mediated by β-glucuronidase enzyme. This releases free and active drug in the intestine, which may be reabsorbed and undergo entero-hepatic cycling. Deconjugation is an important process because it often prolongs the pharmacological effects of drugs and produces toxic effects.

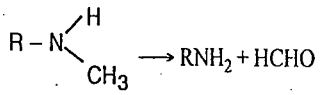
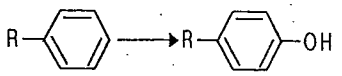
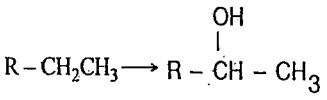
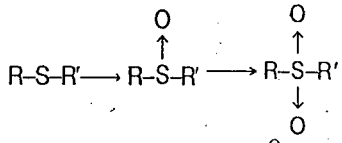
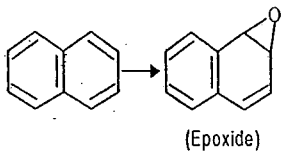
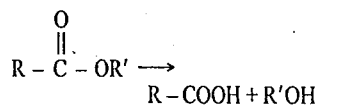
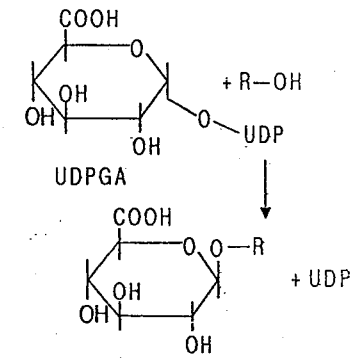
2. Conjugation with sulphate/Sulphation

Conjugation with sulphate (sulphate conjugation, sulphoconjugation, sulphation) is similar to glucuronidation but is catalyzed by non-microsomal enzymes and occurs less commonly. The endogenous donor of the sulphate group is 3'-phosphoadenosine-5'-phosphosulphate (PAPS) and enzyme catalysing the reaction is sulphotransferase. The conjugates of sulphate are referred to as sulphate ester conjugates or ethereal sulphates. Sulphate esters are polar and readily excreted in urine. Unlike glucuronide conjugation, sulphoconjugation in mammals is less important because the PAPS donor that transfers sulphate to the substrate is easily depleted. Capacity for sulphate conjugation is limited in pigs. However in cats, where glucuronidation is minimum, sulphate conjugation is important. Functional groups capable of forming sulphate conjugates include phenols, alcohols, arylamines, N-hydroxylamines and N-hydroxyamides. Drugs undergoing sulphate conjugation include chloramphenicol, phenols, and adrenal and sex steroids. Aging does not affect the process of sulphation.

3. Conjugation with methyl group/Methylation

Conjugation with methyl group (Methyl conjugation or methylation) involves transfer of a methyl group ($-CH_3$) from the cofactor S-adenosyl methionine (SAM) to the acceptor substrate by various methyl transferase enzymes. Methylation reaction is of lesser importance for drugs, but is more important for

Table 4.8. Important drug biotransformation reactions.

Type of reaction	General reaction	Enzyme	Examples
PHASE I REACTIONS			
1. Oxidation			
a. Dealkylation			
i. N-Dealkylation		M	Morphine, diazepam, imipramine.
ii. O-Dealkylation	$ROCH_3 \longrightarrow ROH + HCHO$	M	Codeine, phenacetin.
b. Desulphuration	$RSH \longrightarrow ROH$	M	Thiopental, parathion, malathion.
c. Hydroxylation			
i. Aromatic hydroxylation		M	Phenytoin, propranolol.
ii. Aliphatic hydroxylation		M	Valproic acid, ibuprofen.
d. Oxidation			
i. N-Oxidation	$RNH_2 \longrightarrow RNH-OH$	M	Chlorphenamine, nicotine.
ii. S-Oxidation		M	Chlorpromazine, phenothiazine.
e. Epoxidation (Epoxide formation)	 (Epoxide)	M	Heptachlor, carbamazepine.
f. Deamination	$R-CH_2NH_2 \longrightarrow RCHO + NH_3$	M	Amfetamine, propranolol.
g. Alcohol oxidation	$R-CH_2-OH \longrightarrow R-CHO$	M, N	Ethanol, methanol.
h. Aldehyde oxidation	$R-CHO \longrightarrow R-COOH$	N	Formaldehyde
2. Reduction			
a. Azo-reduction	$RN=NR' \longrightarrow RNH_2 + H_2NR'$	M	Prontosil.
b. Nitro-reduction	$RNO_2 \longrightarrow R-NH_2$	M, N	Parathion, chloramphenicol.
3. Hydrolysis			
a. Hydrolysis of esters		M, N	Procaine, acetylcholine, suxamethonium.
b. Hydrolysis of amides		M, N	Lidocaine,
PHASE II REACTIONS			
1. Conjugation with glucuronic acid (Glucuronidation)		M	Paracetamol, morphine.

Contd...

Type of reaction	General reaction	Enzyme	Examples
2. Conjugation with sulphate (Sulphation)	$PAPS + ROH \longrightarrow RO-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-OH$	N	Paracetamol, salbutamol, aniline.
3. Conjugation with methyl group (Methylation)	$SAM + R-XH \longrightarrow R-X-CH_3 + SAH_C$	N	Morphine, α-methyl dopa, isoprenaline.
4. Conjugation with glutathione	$GSH + RX \longrightarrow R-S-G + H^+ + X^-$	N	Paracetamol, etacrynic acid.
5. Conjugation with acetyl group (Acetylation)	$RNH_2 + CH_3-\overset{\overset{O}{\|}}{C}-SCoA \longrightarrow R-NH-\overset{\overset{O}{\|}}{C}-CH_3$	N	Sulphanilamide, histamine, isoniazid.
6. Conjugation with glycine (Amino acid)	$R-COOH + CoASH \longrightarrow R-\overset{\overset{O}{\|}}{C}-SCoA + NH_2-CH_2COOH \longrightarrow R-\overset{\overset{O}{\|}}{C}-NHCH_2COOH + CoASH$	N	Salicyclic acid, cholic acid, nicotinic acid.
7. Conjugation with thiosulphate	$S_2O_3^{-2} + CN^- \longrightarrow SCN^- + SO_3^{-2}$	N	Cyanide.

Abbreviations : UDPGA = uridine diphosphate-α-D-glucuronic acid; UDP = uridine diphosphate; PAPS = 3′-phosphoadenosine-5′-phosphosulphate; PAP = 3′-phosphoadenosine-5′-phosphate; SAM = S-adenosyl-L-methionine; SAH_C = S-adenosyl-L-homocysteine; GSH = glutathione; M = microsomal; N = non-microsomal.

biosynthesis (e.g. epinephrine and melatonin) and inactivation (e.g. histamine) of endogenous amines. Occasionally the metabolites formed by methylation are not polar or water-soluble and may possess equal or greater activity than the parent compound (e.g. epinephrine synthesised from norepinephrine).

4. Conjugation with glutathione and mercapturic acid formation

Conjugation with glutathione (Glutathione conjugation) and mercapturic acid formation is a minor but important metabolic pathway in animals. Glutathione (GSH, G=glutathione and SH = active –SH group) is a tripeptide having glutamic acid, cysteine and glycine. It has a strong nucleophilic character due to the presence of a –SH (thiol) group in its structure. Thus it conjugates with electrophilic substrates, a number of which are potentially toxic compounds, and protects the tissues from their adverse effects (highly electrophilic substrates have a tendency to react with tissue nucleophilic groups and precipitate toxicity from tissue necrosis, carcinogenesis, mutagenesis, etc.) The interaction between the substrate and the GSH is catalysed by enzyme glutathione-S-transferase, which is located in the soluble fraction of liver homogenates. The glutathione conjugate is either excreted as such in the bile or further metabolised to form mercapturic acid conjugate that is excreted in the urine.

5. Conjugation with acetyl group/Acetylation

Conjugation with acetyl group (Acetylation) is an important metabolic pathway for drugs containing the amino groups. The cofactor for these reactions is acetyl coenzyme A and the enzymes are non-microsomal N-acetyl transferases, located in the soluble fraction of cells of various tissues. Acetylation is not a true detoxification process because occasionally it results in decrease in water solubility of an amine and, thus, increases in its toxicity (e.g. sulphonamides). Acetylation is the primary route of biotransformation of sulphonamide compounds. Dogs and foxes do not acetylate the aromatic amino groups due to deficiency of arylamine acetyltransferase enzyme. Aging does not affect metabolism of drugs by process of acetylation.

6. Conjugation with amino acids

Conjugation with amino acids occurs to a limited extent in animals because of limited availability of amino acids in body. The most important reaction involves conjugation with glycine. Conjugation with other amino acids like glutamine in man and ornithine in birds is also seen. Examples of drugs forming glycine or glutamine conjugates are salicylic acid, nicotinic acid and cholic acid.

7. Conjugation with thiosulphate

Conjugation with thiosulphate is an important reaction in the detoxification of cyanide. Conjugation of cyanide ion involves transfer of sulphur atom from the thiosulphate to the cyanide ion in presence of enzyme rhodanese to form inactive thiocyanate. Thiocyanate formed is much less toxic than the cyanide (true detoxification) and it is excreted in urine.

Induction of Drug Metabolising Enzymes

Several drugs and chemicals have the ability to increase the drug metabolizing activity of certain enzymes referred to as enzyme induction. These drugs known as enzyme inducers mainly interact with DNA and cause an increase in liver weight, proliferation of the smooth endoplasmic reticulum and enhance synthesis of microsomal enzyme proteins, especially cytochrome P-450 and

glucuronyl transferase. As a result, there is enhanced metabolism of those endogenous substances (e.g. sex steroids) and drugs those are metabolized by microsomal enzymes. Some drugs (e.g. carbamazepine and rifampicin) may stimulate their own metabolism, the phenomenon being called auto-induction or self induction. Since different cytochrome P-450 isoenzymes are involved in the metabolism of different drugs, enzyme induction by one drug affects metabolism of only those drugs which are substrate for the induced isoenzyme. Some drugs like phenobarbital may affect metabolism of a large number of endogenous substances and drugs because they induce isoenzymes like CYP3A and CYP2D6 which act on many drugs.

Enzyme inducers are generally lipid-soluble compounds with relatively long plasma half-lives. Repeated administration of inducers for a few days (3 to 10 days) is often required for enzyme induction and on stoppage of drug administration, the enzymes return to their original value over 1 to 3 weeks. Non-microsomal enzymes are not known to be induced by any drug or chemical.

Clinical importance of enzyme induction

Microsomal enzyme induction by drugs has great clinical importance because:

a. It reduces efficacy and potency of drugs metabolised by these enzymes.
b. It reduces plasma half-lives and duration of action of drugs.
c. It enhances drug tolerance.
d. It increases drug toxicity by enhancing concentration of metabolite, if metabolite is toxic. It increases chances of drug interactions.
e. It alters physiological status of animal due to altered metabolism of endogenous compounds like sex steroids.

Inhibition of Drug Metabolising Enzymes

Contrary to the metabolising enzyme induction, several drugs or chemicals have the ability to decrease the drug metabolising activity of certain enzymes known as enzyme inhibition. Enzyme inhibition can be either non-specific of microsomal enzymes or specific of some non-microsomal enzymes (e.g. monoamine oxidase, cholinesterase and aldehyde dehydrogenase). The inhibition of hepatic microsomal enzymes mainly occurs due to administration of hepatotoxic agents, which cause either rise in the rate of enzyme degradation (e.g. carbon tetrachloride and carbon disulphide) or fall in the rate of enzyme synthesis (e.g. puromycin and dactinomycin). Nutritional deficiency, hormonal imbalance or hepatic dysfunction also inhibit microsomal enzymes indirectly. Inhibition of non-microsomal enzymes with specific function usually results when structurally similar compounds compete for the active site on the enzymes. Such an inhibition is usually rapid

Table 4.9: List of selected drug metabolising enzyme inducers and inhibitors, and drugs commonly affected by them.

Enzyme inducers/inhibitors	Examples of drugs affected
Enzyme Inducers	**Drugs with increased metabolism**
Microsomal enzymes	
Barbiturates (e.g. phenobarbital)	Warfarin, phenytoin, corticosteroids, phenothiazines, digitoxin, estradiol, testosterone, barbiturates, Ca^{++} channel blockers.
Phenytoin	Warfarin, corticosteroids, theophylline, tolbutamide, quinidine, propranolol.
Rifampicin	Warfarin, corticosteroids, phenytoin, digitoxin, theophylline, propranolol, Ca^{++} channel blockers.
Carbamazepine	Corticosteroids, estradiol, doxycycline, haloperidol.
Ethanol	Warfarin, barbiturates, ethanol, phenytoin.
Griseofulvin	Warfarin.
Microsomal enzymes	
Cimetidine	Diazepam, phenytoin, propranolol, theophylline, lidocaine, quinidine, warfarin, antidepressants.
Enzyme Inhibitors	**Drugs with decreased metabolism**
Microsomal enzymes	
Erythromycin	Warfarin, theophylline, ciclosporin, carbamazepine.
Quinolones	Theophylline.
Chloramphenicol	Phenytoin, tolbutamide, warfarin.
Sodium valproate	Phenytoin, phenobarbital, primidone.
Non-microsomal enzymes	
MAO-Inhibitors (e.g. tranylcypromine)	Tyramine, catecholamines, barbiturates, pethidine, tricyclic antidepressants.
ChE-Inhibitors (e.g. physostigmine)	Suxamethonium, procaine.
Xanthine oxidase inhibitors (e.g. allopurinol)	6-Mercaptopurine, azathioprine.
Aldehyde dehydrogenase inhibitors (e.g. disulfiram)	Ethanol, phenytoin, some benzodiazepines.

Abbreviations: MAO = monoamine oxidase; ChE = cholinesterase.

(a single dose of inhibitor may be sufficient) and clinically more important than the non-specific microsomal enzyme inhibition.

Enzyme inhibition generally results in depressed metabolism of drugs. As a result, the plasma half-life, duration of action, and efficacy as well toxicity of the object drug (whose metabolism has been inhibited) are significantly enhanced. In case the drug undergoes hepatic first-pass effect, the bioavailability and toxicity of the drug will be markedly increased in presence of enzyme inhibition. Enzyme inhibition may also produce undesirable drug interactions. In therapeutics, some specific enzyme inhibitors like monoamine oxidase inhibitors, cholinesterase inhibitors and angiotensin converting enzyme (ACE) inhibitors are purposely used for producing desirable pharmacological actions.

First-Pass Effect/First-Pass Metabolism

First-pass effect (First-pass metabolism or pre-systemic metabolism) may be defined as the loss of drug through biotransformation before it enters systemic circulation. This may occur during passage of drug through intestine or liver for first time (therefore called first-pass effect/metabolism) after oral administration.

a. **Intestinal first-pass effect:** In this type, drugs are metabolised in the gastrointestinal tract by enzymes present either in gut mucosa or gut lumen before they are absorbed. Microorganisms present in the GI tract also inactivate some drugs. Such drugs are not suitable by oral administration due to poor bioavailability (e.g. catecholamines).

b. **Hepatic first-pass effect:** In this type, drugs are suitably absorbed across the GI tract and enter portal circulation, but they are rapidly and significantly metabolised during the first passage through the liver. Such drugs are also not/less suitable by oral administration due to their failure to reach systemic circulation and thus having poor bioavailability. Examples of drugs undergoing significant hepatic first-pass effect include propranolol, lidocaine and nitroglycerine.

EXCRETION

Excretion may be defined as a process by which drugs and/or their metabolites are irreversibly transferred from the body to external environment. Excretion is thus important in termination of pharmacological action of drugs. Excretory organs (except lungs) excrete water soluble and ionised compounds more effectively than the lipid soluble and unionised compounds. Lipid soluble drugs have to be converted into water soluble forms by process of metabolism to facilitate their excretion from the body.

ROUTES OF EXCRETION

Drugs are excreted in their unchanged form or as metabolites by several routes including the kidneys, lungs, biliary system, intestine, etc. Excretion of drugs by kidneys, the principal organ of excretion, is called renal excretion and excretion by organs other than kidneys is known as extra-renal or non-renal excretion.

I. Renal Excretion of Drugs

Renal excretion is the most important route of drug excretion. Agents which are water soluble, non-volatile and small in molecular size are excreted in the urine. The amount of drug or its metabolite ultimately excreted in urine depends on the sum total of three processes: glomerular filtration, tubular secretion and tubular reabsorption (Figure 4.7). The first two processes remove drugs from plasma, whereas the third process reabsorbs drugs and returns them to plasma.

Glomerular filtration: It is a non-selective and unidirectional process by virtue of which all non-protein bound drugs (whether lipid soluble or water soluble, unionised or ionised) are filtered through glomerular membranes into the renal tubules. Drugs which are protein bound (Mol. wt. > 65,000) are not passed into glomerular filtrate because of their big size. The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries. Therefore, glomerular filtration of a drug depends mainly on its plasma protein binding and renal blood flow. If the blood flow to kidneys and the glomerular filtration is decreased due to any reason (e.g. hypovolaemia and hypotension), then excretion of drugs in urine may also get decreased. A drug that is passed via glomerulus into the glomerular filtrate may either be reabsorbed (see tubular reabsorption) or excreted in the urine.

Tubular secretion: It is a carrier-mediated process by which some drugs and/or their metabolites are actively secreted from the blood into the tubular lumen. This transport is against concentration or electrochemical gradient, energy dependent, capacity limited and saturable process. Binding to plasma proteins usually does not hinder tubular secretion of a drug because as the free drug is removed and transported across the tubular epithelium, immediate dissociation of the drug-protein complex occurs and the free form is again secreted. Active secretion is unaffected by changes in pH, but is dependent on the renal blood flow.

Two active tubular systems have been identified for organic electrolyte drugs, one for acidic drugs (e.g. penicillins, salicylic acid and furosemide) and other for basic drugs (e.g. morphine, quinidine and catecholamines). Both systems are relatively non-selective and independent of each other. Both systems are bidirectional i.e. they can transport their substrates from blood to tubular fluid and *vice-versa*. However for most drugs and metabolites, secretion into the tubular lumen predominates, whereas endogenous substances like uric acid are predominantly reabsorbed. Drugs having similar ionic charge and utilising same carrier transport usually compete with each other. This can prolong the elimination of a drug that has lesser affinity for the carrier site, thereby increasing its duration of action. (e.g. probenecid prolongs the duration of penicillin G by decreasing its tubular excretion).

Tubular reabsorption: It is a process by which drugs and/or their metabolites are transported from tubular fluid back into the capillary network. It generally occurs after glomerular filtration of drugs and takes place all along the renal tubule. Contrary to the tubular secretion, reabsorption results in increase in the half-lives of drugs. Tubular reabsorption is further of two types: passive and active tubular reabsorption.

Passive tubular reabsorption: It is a passive process by which lipid soluble and unionised drugs are reabsorbed after they are filtered at the glomerulus. The driving force for such a process is the concentration gradient established by reabsorption of water along with sodium and other inorganic ions (as glomerular filtrate traverses the tubules, water is reabsorbed into blood stream and filtrate becomes concentrated, thus creating a concentration gradient of solutes between tubular fluid and plasma). Because most drugs are weak acids or weak bases, the pK_a of the drug and the pH of the urine in tubular fluid determine the degree of ionisation, and hence extent of diffusion through the lipoidal tubular membrane. According to the pH-partition hypothesis, weak

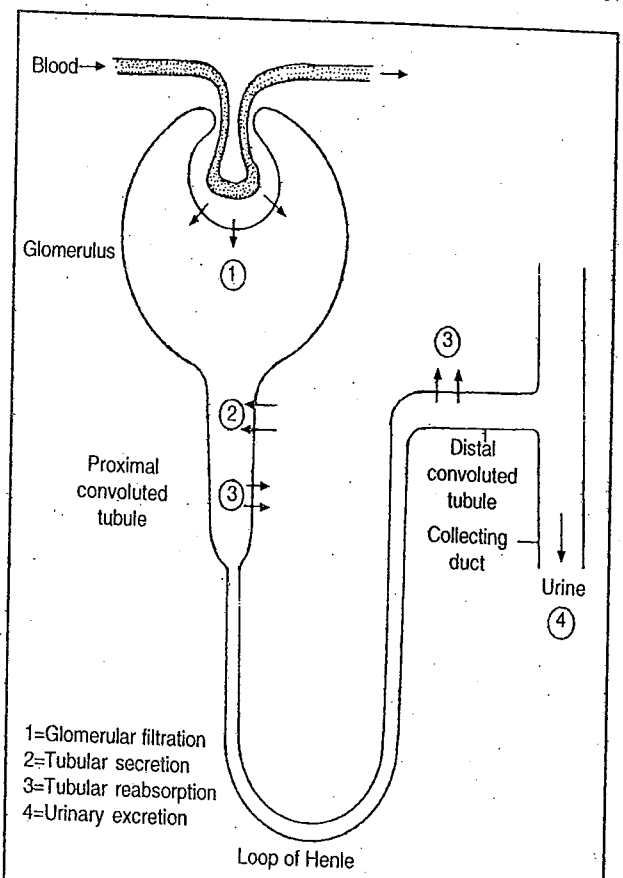


Fig. 4.7. Diagrammatic representation of renal excretion.

bases ionise more and are absorbed less in acidic urine, whereas weak acids ionise more and are absorbed less in alkaline urine. Thus acidification or alkalinisation of urine may alter the rate of excretion of such drugs because of ion-trapping in the tubular fluid. This principle is utilised for facilitating elimination of some drugs in cases of drug overdosage or poisoning. For example, urine is acidified in poisoning with basic drugs (e.g. morphine and amfetamine), and alkalinised in poisoning with acidic drug (e.g. barbiturates and salicylates). Variation in normal urine pH in veterinary species (carnivores = 5.5 to 7.0, herbivores = 7.0 to 8.0) may also affect the degree of ionisation and hence the passive reabsorption of drugs.

Active tubular reabsorption: Active tubular reabsorption is commonly seen with some endogenous substances or nutrients such as glucose, amino acids, etc., which the body needs to conserve. Very few drugs (e.g. oxopurinol) are known to undergo active renal tubular reabsorption.

II. Extra-Renal Excretion of Drugs

1. Biliary excretion

Biliary excretion is an important route of drug removal in which drugs and/or their metabolites are excreted by hepatocytes into the bile canaliculi and ultimately into the duodenum via the bile. Biliary excretion of drugs may occur both passively and actively (mainly). Biliary excretion is mainly determined by polarity of the substance – greater the polarity, better the biliary excretion. Metabolites, particularly water-soluble conjugates, are more excreted in bile than the parent compounds. Relatively large molecules are preferentially eliminated in the bile. Therefore, a metabolic reaction that greatly increases the polarity and molecular weight of the drug favours biliary excretion of the metabolite. Accordingly, glucuronide conjugates are mainly and glutathione conjugates are exclusively excreted via the bile. For a drug to be excreted unchanged in the bile, it must have highly polar functional groups. (e.g. cromoglicic acid). Non-ionised drugs should also be highly polar for biliary excretion (e.g. cardiac glycosides).

After biliary excretion, some of the conjugated metabolites, especially glucuronide conjugates, are deconjugated by intestinal bacteria and the released lipid soluble drugs are reabsorbed into the circulation. The reabsorbed drugs are again carried to the liver for resecretion via bile into the intestine. This phenomenon of drug cycling between intestine and liver is called 'enterohepatic cycling or enterohepatic circulation of drugs' (Figure 4.8). Such a recycling process continues until the drug is further biotransformed in liver or is excreted in urine, or both. The drugs which are excreted via bile in the intestine but do not undergo enterohepatic cycling are finally excreted in faeces. The enterohepatic cycling is an important phenomenon that prolongs the duration of action of several drugs (e.g. digoxin, chloramphenicol and some benzodiazepines).

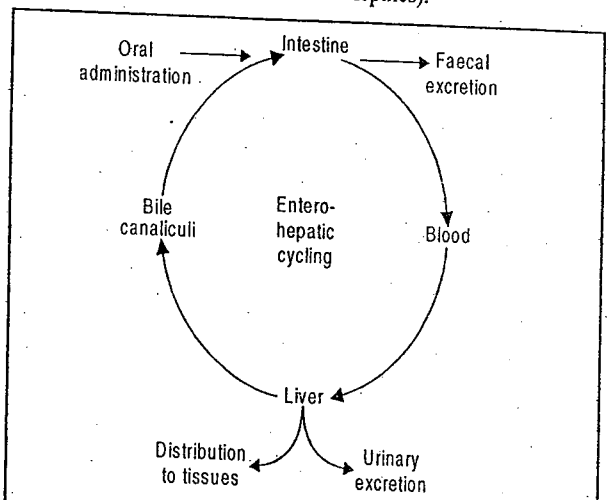


Fig. 4.8. Entero-hepatic cycling of drugs.

2. Pulmonary excretion

Gases and volatile substances such as inhalation anaesthetics are absorbed and excreted through the lungs by simple diffusion. Generally lipid soluble and unchanged drugs are excreted via lungs. Gaseous anaesthetics which are not very soluble in blood such as nitrous oxide are excreted rapidly. Compounds like alcohols which have high solubility in blood and tissues are excreted slowly by the lungs. Factors influencing pulmonary excretion of drugs include rate of respiration, pulmonary blood flow, solubility of drug, etc.

3. Mammary excretion

Excretion of drugs in milk is a minor route of drug elimination, but is important because drugs given to the mother can affect the suckling young ones. Most drugs enter milk by passive diffusion and are dependent upon lipid solubility, molecular weight and degree of ionization. As milk is acidic (pH 6.5 to 7.0) relative to plasma (pH 7.4), weakly basic drugs concentrate more in milk. The opposite is true for weakly acidic drugs. Since milk contains proteins, some drugs (e.g. sulphonamides) excreted in milk can

bind to milk proteins. Lipophilic drugs and toxicants (e.g. DDT) may bind to milk fat and get excreted along with milk. Discolouration of teeth with tetracyclines, sedation with phenobarbital and immune suppression with doxorubicin in human infants are examples of adverse effects noted due to drug excretion in the milk.

4. Salivary excretion

Excretion of drugs in saliva is of lesser importance. It occurs primarily by passive diffusion process and, therefore, is predictable on the basis of the pH-partition hypothesis. Some drugs (e.g. penicillin and phenytoin) are actively excreted in saliva. Drugs excreted in saliva can undergo cycling in a fashion similar to entero-hepatic cycling. Some drugs (e.g. phenytoin and penicillin) are also actively secreted in saliva. Salivary excretion is important in ruminants receiving parenteral antimicrobial drugs because swallowing of such drugs through saliva adversely affects the rumen microflora and may upset the digestive process. In humans, the salivary secretion is responsible for side effects such as black hairy tongue in patients receiving antibiotics and gingival hyperplasia due to phenytoin.

5. Gastro-intestinal excretion

Excretion of drugs into the GI tract is a minor route of drug elimination. The process is reverse of GI absorption of drugs in which drugs may diffuse from the blood into the lumen of GI tract. It usually occurs after parenteral administration of drugs when the concentration gradient for passive diffusion is favourable. Orally administered drugs can also be absorbed and then excreted in the GI tract. Drugs excreted in the GI tract may undergo recycling (e.g. morphine) or excreted in faeces. Weak organic bases, administered parenterally, diffuse into the rumen of cattle and sheep, and are exposed to the metabolic action of micro-organisms.

6. Other routes of excretion

Insignificant amounts of some drugs (e.g. salicylic acid, alcohol and heavy metals) are excreted through skin via sweat. Drugs can also be excreted via the lachrymal fluid, intestinal fluid, genital secretions and trachea-bronchial secretions. The heavy metals (e.g. arsenicals) may be detected in hair and nails.

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CHAPTER 5 PRINCIPLES AND BASIC CONCEPTS OF PHARMACOKINETICS

Pharmacokinetic principles
Plasma drug concentration-time profile
Orders of pharmacokinetic processes
  Zero-order process/Zero-order kinetics
  First-order process/First-order kinetics
  Mixed-order process/Mixed-order kinetics
Pharmacokinetic models
  Compartmental models
    One-compartment open model
    Two-compartment open model
    Three-compartment open model
  Non-compartmental models
  Physiological models
Determinants of pharmacokinetics
  Absorption
    Bioavailability
    Area under curve
  Distribution
    Volume of distribution
  Elimination
    Half-life
    Clearance
Design of dosage regimen
  Dose
  Dosing interval
Steady-state concentration
  Multiple dosing
  Accumulation
  Fluctuation
  Clinical applications
  Intravenous infusion

The previous chapter has described the four processes of drug's fate in body namely absorption, distribution, metabolism and excretion. These processes are often discussed separately and in sequence for convenience, but in body they interact and overlap in time. As the study of the rate and extent of various pharmacokinetic parameters individually is difficult and complex, an alternative approach has been adopted to study them from plasma drug concentration-time data by using certain pharmacokinetic principles.

PHARMACOKINETIC PRINCIPLES

Pharmacokinetic principles relate specifically to the variations in drug concentration as a result of pharmacokinetic processes i.e. absorption, distribution and elimination with time. Accordingly, pharmacokinetics may also be defined as the mathematical description of concentration changes of drug as a function of time. The knowledge of pharmacokinetic principles is essential in therapeutics because it shows relationship between the pharmacological/toxicological effect of a drug and the concentration of drug in body. Pharmacokinetic principles are useful in determination of following factors:

a. The extent and rate to which a pharmacokinetic parameter (ADME) occurs in body.
b. The dose and dosing schedule of a drug and its modification according to individual needs.
c. The drug concentrations which produce therapeutic and toxic effects.
d. The drug concentrations in various body fluids and tissues, and the accumulation of drug or its metabolites in body.
e. The half-life and duration of action of a drug.
f. The effect of disease state on various pharmacokinetic parameters.
g. The withdrawal time for meats, eggs and dairy products, when a drug is administered to food producing animals.
h. The nature and extent of drug interactions.

PLASMA DRUG CONCENTRATION-TIME PROFILE

The speed of onset, intensity and duration of a pharmacological effect depend on the concentration of drug at its site(s) of action. As it is difficult to collect tissue samples, the time course of drug concentration at its site of action is approximated by measuring drug concentrations in plasma, which can be measured with accuracy. The plasma drug concentration-time curve is obtained by measuring the concentrations of drug in plasma taken at different time intervals after administration of the drug and then plotting the concentrations of drug in plasma (Y-axis) versus the corresponding time at which the plasma samples were collected (X-axis).

If a drug is administered by oral or extravascular (IM, SC, IP, etc.) route, it slowly enters the systemic circulation and plasma drug concentration gradually rises to a maximum (peak) level. As the drug is being absorbed into blood, it is distributed to body tissues and may also simultaneously being eliminated. The ascending portion of the curve to left of peak represents the

absorption phase because in this phase rate of absorption is always greater than rate of distribution and elimination. Generally the distribution phase of a non-intravenous dose is masked by the absorption phase. The descending section of the curve to right of peak generally represents the elimination phase because in this phase rate of elimination always exceeds rate of absorption. The rate or velocity with which absorption and elimination processes occur is given by the respective slopes of curve and are expressed by absorption rate constant (K_a) and elimination rate constant (β), respectively. The typical plasma concentration curve obtained after a single oral dose of a drug, and the relationship of the drug concentration-time curve and various pharmacological parameters are depicted in Figure 5.1.

a. ***Minimum effective concentration (MEC):*** It is the minimum concentration of a drug in plasma required to produce the desirable pharmacological/therapeutic response. In case of antimicrobial drugs, the term minimum inhibitory concentration (MIC) is used, which may be defined as the minimum concentration of an antimicrobial agent in plasma required to inhibit the growth of microorganisms.

b. ***Maximum safe concentration (MSC) or Minimum toxic concentration (MTC):*** It is the concentration of a drug in plasma above which toxic effects are produced. Concentrations of drug above MSC are said to be in toxic level. The drug concentrations between MEC and MSC represent the therapeutic range.

c. ***Maximum plasma concentration/Peak plasma concentration (C_{max} or C_{pmax}):*** It is the maximum or highest concentration of a drug in plasma. The maximum plasma concentration depends on the administered dose and rates of absorption (absorption rate constant, K_a) and elimination (elimination rate constant, β). The peak plasma concentration represents the point of time when absorption rate equals elimination rate of the drug. It is often expressed as µg/ml.

d. ***Area under curve (AUC):*** It is the total integrated area under the plasma drug concentration–time curve. It expresses the total amount of drug that comes into systemic circulation after its administration.

e. ***Peak effect:*** It is the maximal or peak pharmacological effect produced by a drug. It is generally observed at peak plasma concentration.

f. ***Time of maximum concentration/Time of peak concentration (t_{max}):*** It is the time required for a drug to reach peak concentration in plasma. The faster the absorption rate, the lower is the t_{max}. It is also useful in assessing the efficacy of drugs used to treat acute conditions (e.g. pain) which can be treated by a single dose. The t_{max} is expressed in hours.

g. ***Onset of action:*** It is the beginning of pharmacological response produced by a drug. It occurs when the plasma drug concentration just exceeds the MEC.

h. ***Onset time:*** It is the time required for a drug to start producing pharmacological response. It usually corresponds to the time for the plasma concentration to reach MEC after administration of drug.

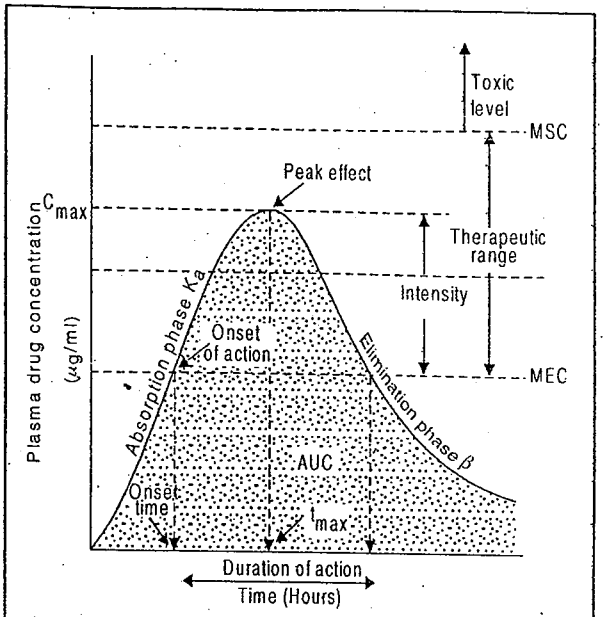


Fig. 5.1. Plasma drug concentration-time profile after oral administration of a single dose of a drug.
Abbreviation : C_{max} = Maximum/Peak plasma concentration, MEC = minimum effective concentration; MSE = maximum safe concentration; t_{max} = time of peak concentration; AUC = area under curve; K_a = absorption rate constant ; β = elimination rate constant.

i. ***Duration of action:*** It is the time period for which pharmacological response is produced by a drug. It usually corresponds to the duration for which the plasma concentration of drug remains above the MEC level.

Orders of Pharmacokinetic Processes

The rate at which various pharmacokinetic processes (ADME) occur is affected by the amount of drug in body. The manner in which concentration of drug influences the rate of a process is called the order of process. The three commonly encountered rate processes in pharmacokinetics are zero order process, first order process and mixed order process.

a. **Zero-order process/Zero-order kinetics:** Zero-order process (zero-order kinetics or constant-rate kinetics) may be defined as a pharmacokinetic process whose rate is independent of the concentration of drug i.e. the rate of pharmacokinetic process remains constant and cannot be increased further by increasing the concentration of drug. In other words, in the zero-order kinetics a fixed amount/quantity of drug is processed per unit time. Examples of zero-order kinetics include absorption, distribution, metabolism and excretion of drugs by carrier mediated transport under saturated conditions. Administration of drugs by a constant rate IV infusion or by controlled delivery system (e.g. implants) also tends to follow zero-order kinetics. Examples of drugs which follow zero-order kinetics are alcohol, phenytoin, salicylates, etc.

As in the zero order kinetics a constant/fixed amount of drug is eliminated per unit of time, the half-life of drug undergoing zero-order elimination is not constant but is proportional to the concentration of drug in the plasma. Therefore the zero-order half-life rises with increase in

drug concentration and declines with decrease in drug concentration. Special care is needed while increasing dose of drugs which follow zero-order kinetics to avoid adverse drug effects.

b. **First-order process/First-order kinetics:** First-order process (First-order kinetics or linear kinetics) may be defined as a pharmacokinetic process whose rate is directly proportional to the concentration of drug i.e. greater the concentration, faster the process. In contrast to the zero-order kinetics, the first-order kinetics processes a fixed fraction of drug per unit time. It is because of such a proportionality between the rate of process and the concentration of drug that the first-order process is said to follow linear kinetics i.e. log-plasma concentration-time curve is linear. The first-order pharmacokinetic processes are non-saturable over a wide range of plasma concentration. Most drugs follow first-order kinetics in absorption, distribution, metabolism and excretion.

As in the first-order kinetics a constant fraction of drug is eliminated per unit time, the half-life of a drug undergoing first-order elimination remains constant and independent of the drug concentration in plasma.

c. **Mixed-order process/Mixed-order kinetics:** Mixed-order process (Mixed order kinetics, non-linear kinetics or dose-dependent kinetics) may be defined as a pharmacokinetic process whose rate is a mixture of both zero-order and first-order processes. The mixed order process follows first order kinetics at lower concentration and zero-order kinetics at high concentration of drug. This type of kinetics is generally observed at increased or multiple doses of some drugs. The phenomenon is mainly seen when a particular pharmacokinetic process involves presence of a carrier or enzyme, which is substrate specific, has definite capacity, and gets saturated at high drug concentration. Since in such cases deviation from an originally linear pharmacokinetic profile is observed, the rate process of such a drug is called non-linear kinetics. Mixed-order kinetics has been observed in the absorption (e.g. vitamin C), distribution (e.g. naproxen), and elimination (e.g. riboflavin) of some drugs.

PHARMACOKINETIC MODELS

Drug movement within the body is a complex process and various pharmacokinetics events (ADME) often happen simultaneously. In order to describe the complex and overlapping pharmacokinetic processes, certain assumptions have been made regarding the movement of drug and some pharmacokinetic models were developed. These pharmacokinetic models provide concise means of expressing mathematically or quantitatively the time course of drug(s) throughout the body and help compute meaningful pharmacokinetic parameters. Presently, three types of models are used in the pharmacokinetic analysis of drugs: compartmental models, non-compartmental models and physiological models.

I. Compartmental Models

Compartmental analysis is the traditional and most commonly used approach to the pharmacokinetic characterisation of a drug. In this model, body is considered to be composed of several compartments which communicate reversibly with each other. As infinite number of compartments can exist in body (each organ, tissue or body fluid can form a separate compartment), tissues having approximately similar drug distribution characteristics are pooled to form a kinetically homogeneous hypothetical compartment. Such compartments are, thus, only mathematical entities and have no physiological or anatomical meaning. Generally model consisting of one, two or at the most three compartments arranged parallel to each other are used to describe kinetics of most drugs. These compartments are called open models because there is no restriction to the movement of drugs between compartments as drugs freely move from one compartment to the other. It is also assumed that the rate of drug transfer between compartments and the rate of drug elimination from compartments follow first-order or linear kinetics. Compartmental characteristics of a drug are best understood by giving it as intravenous bolus and observing the manner in which the plasma concentration declines with time. The number of exponents required to describe such a plasma level-time profile determines the number of

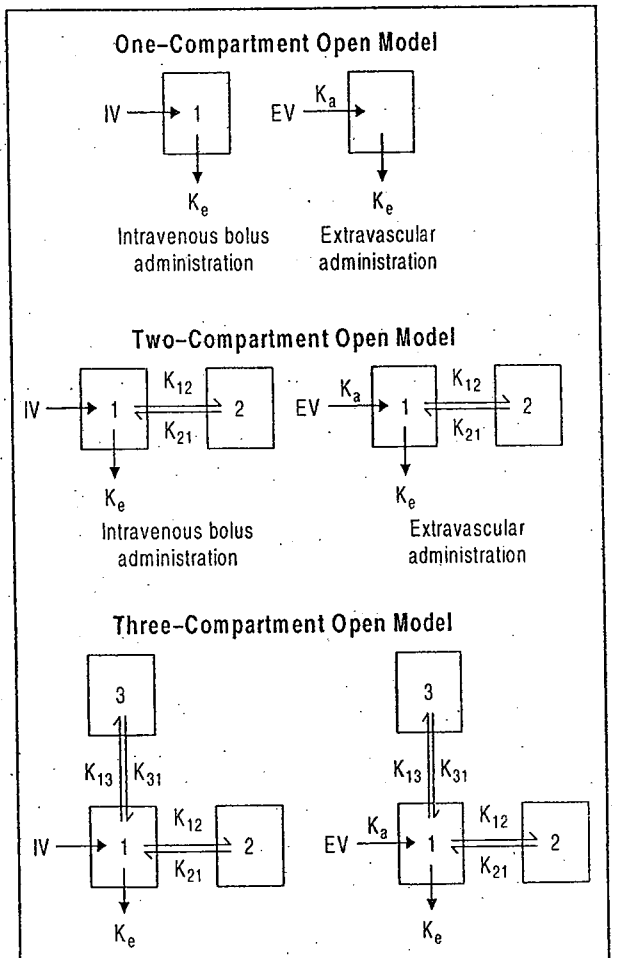


Fig. 5.2. Schematic representation of various compartment models. **Abbreviation :** IV = intravenous; EV = extravenous ; K_a = first order absorption rate constant; K_e = first order elimination (from central compartment) rate constant ; K_{12} = first order rate constant for drug transfer from central (1) to peripheral (2) compartments ; K_{21} = first order rate constant for drug transfer from peripheral (2) to central (1) compartments ; K_{13} = first order rate constant for drug transfer from central (1) to peripheral (3) compartments ; K_{31} first order rate constant for drug transfer from peripheral (3) to central (1) compartments.

compartments in which a drug will distribute. Various compartment models are depicted in Figure 5.2.

1. One-compartment open model

One-compartment open model is the simplest model, which considers the whole body as a single, kinetically homogeneous unit. In this model, the final distribution equilibrium between the drug in plasma and other body fluids is attained instantaneously and maintained at all times. Any change in the plasma level of drug reflects proportional changes in tissue drug concentrations. The one-compartment open model applies to only those drugs which distribute rapidly throughout the body. The model is generally used to describe plasma levels following administration of a single dose of a drug, and can be observed following either intravenous bolus or extravascular administration of drugs.

Intravenous bolus administration: When a drug that distributes instantaneously in body is given as rapid IV bolus, the entire dose of drug enters the body and distributed immediately via circulation to all tissues. In such a situation, the drug concentration-time curve will be obtained as a straight line on semi-logarithmic paper showing monophasic exponential decline (Figures 5.3 and 5.4). In this model, the decline in plasma drug concentration is only due to the elimination of drug from body, the phase being called elimination phase. The distribution phase is normally neglected in calculations because distribution is so rapid that it cannot be shown on graph. The extrapolated zero-time intercept of linear elimination phase gives the coefficient B, and the elimination rate constant is given by β. The value of B is an estimate of zero-time concentration of drug in plasma (C_0 or C_p^0).

Extravascular administration: When a drug is administered by extravascular route (e.g. oral, IM and SC), absorption is a pre-requisite for its action. If the drug is distributed according to one compartment open model, the rate of change in drug concentration in the central compartment is described by 2 exponents – K_a an absorption exponent and β an elimination exponent (Figure 5.4).

2. Two-compartment open model

Two-compartment open model assumes that body is composed of two compartments viz. the central compartment and the peripheral compartment. The central compartment (compartment 1) consists of blood and highly perfused organs such as liver, kidneys, lungs, heart and brain and the peripheral compartment (compartment 2) comprises of less perfused tissues like skin, muscles, bone, cartilage, etc. The drug is directly administered (IV route) or absorbed (extravascular route) into the central compartment and various organs in it rapidly equilibrate with it. Elimination of drug also occurs only from central compartment since main organs involved in drug elimination (e.g. liver and kidneys) are located in it. The distribution of drugs from blood (central compartment) to peripheral compartment occurs slowly. It is assumed that drug transfer from central compartment to peripheral compartment, and back from peripheral compartment to central compartment occurs by first-order process and is defined by rate constants denoted by letter k. The subscript indicates the direction of drug movement e.g. k_{12} refers to drug movement from compartment 1 (central compartment) to compartment 2 (peripheral compartment), and reverse for k_{21} (Figure 5.2). The two compartment open model adequately describes the disposition kinetics of most therapeutic agents in humans and animals.

Intravenous bolus administration: After the intravenous bolus administration of a drug that follows two-compartment kinetics, the decline in plasma concentration is biexponential. The plasma concentration declines rapidly in first phase followed by a slow terminal decline as depicted clearly when plasma drug concentration-time curve is plotted on a semi-logarithmic paper. The initial relatively non-linear steep decline in drug concentration is attributed mainly to distribution of drug from the central compartment to the peripheral compartment, and terminal shallow linear decline mainly to elimination of drug from the central compartment. The linear terminal portion of curve is called the elimination phase. By extrapolation of the linear portion, we can find the blood concentration at time 0 (C_0 or C_p^0). If the biexponential curve is resolved into its components by the method of residuals (feathering technique), then distribution curve may also be obtained as a linear curve called the distribution phase. The extrapolated zero-time intercept of linear distribution phase (residual line) is denoted by letter A and the elimination phase by letter B (Figures 5.3 and 5.4). For two compartment model, α is the rate constant for distribution and β is the rate constant for elimination; they are expressed in units of reciprocal time (min^{-1}). In drug disposition studies, the coefficients (A and B) and rate constants (α and β) are calculated from the experimental data by least squares regression analysis. The sum of A and B coefficients gives the initial concentration of drug in the plasma (C_0).

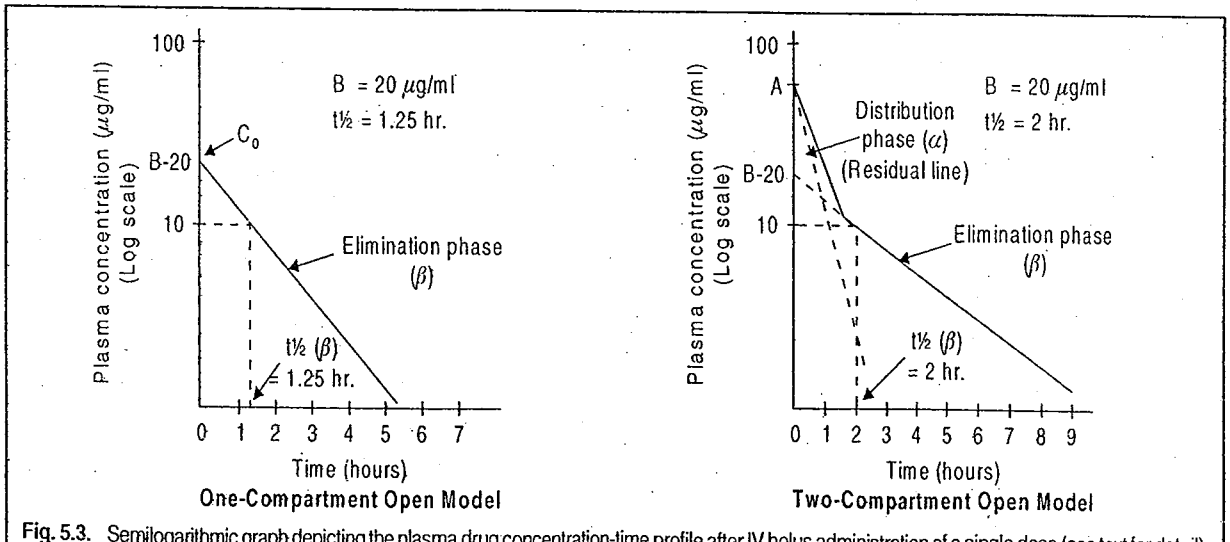


Fig. 5.3. Semilogarithmic graph depicting the plasma drug concentration-time profile after IV bolus administration of a single dose (see text for detail).

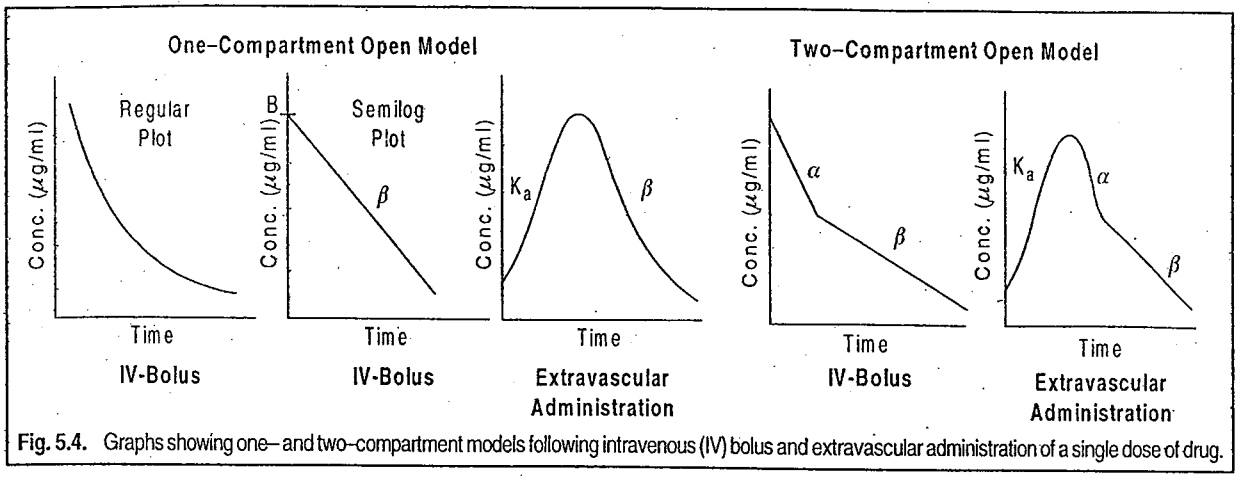


Fig. 5.4. Graphs showing one– and two-compartment models following intravenous (IV) bolus and extravascular administration of a single dose of drug.

Extravascular administration: For a drug that enters the body by an extravascular route (e.g. oral, IM, SC, etc.) and is distributed according to two compartment model, the rate of change in drug concentration in plasma is described by 3 exponents – K_a an absorption exponent, α a distribution exponent and β an elimination exponent (Figure 5.4).

3. Three-compartment open model

The pharmacokinetic behaviour of some drugs, which have high affinity for a particular tissue or undergo redistribution, is best interpreted according to a three compartment open model. In this compartmental model, body is conceived as consisting of three compartments – one central and two peripheral compartments. The central compartment (compartment 1) comprises of plasma and highly perfused organs, whereas peripheral compartments 2 and 3 comprise of moderately (e.g. skin and muscles) and poorly (e.g. bone, teeth, ligaments, hair and fat) perfused tissues respectively. If a drug is administered in body by intravenous bolus injection, it is first distributed immediately into the highly perfused tissues (compartment 1), then slowly into the moderately perfused tissues (compartment 2) and thereafter very slowly to the poorly perfused tissues (compartment 3). If the plasma level-time profile is plotted on a semi-logarithmic graph, it gives a triexponential appearance.

II. Non-Compartmental Models/Non-Compartmental Analysis

Non-compartment analysis is another approach to study the time course of drugs in body. It does not require the assumption of specific compartmental model. It considers the time course of drug concentration in plasma as a statistical distribution curve and derives pharmacokinetic parameters by simple algebraic equations. A detailed description of drug disposition characteristic is not required. The disadvantage of this method is that it often deals with averages and provides limited information regarding plasma drug concentration-time profile.

III. Physiological Models

Physiological models (Perfusion rate-limited models) technique is a newer approach devised to study the time course of drugs in body. The basis of this method is that each major organ system, plus any specialised target site, represents a physiological compartment. Each separate compartment has its own blood flow, tissue volume, uptake process, affinity for drug (i.e. partition coefficient) and elimination process. Thus, the kinetics of the drug is described by a series of flow-related equations, which can be solved following the incorporation of known physiological values (e.g. organ/tissue volume and perfusion rate) and experimental estimates (e.g. tissue to plasma partition coefficients).

The physiological models have some advantages as they are drawn on the basis of known anatomical and physiological data and they present a more realistic picture of drug disposition in various organs and tissues. The physiological models also allow an assessment of the impact of altered physiology (e.g. ageing) or pathology on the drug disposition. More importantly, species differences can be predicted based on known perfusion rates. The only disadvantage of physiological models is obtaining experimental data, which are very exhaustive. Figure 5.5 shows a physiological model where compartments are arranged in a series in a flow diagram.

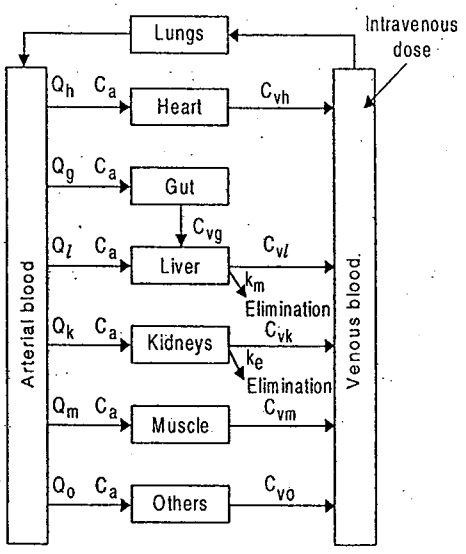


Fig. 5.5. Schematic representation of a physiological pharmacokinetic model. The term Q indicates blood flow to a body region. C_a is the arterial concentration, whereas C_v is the venous concentration. K_m indicates rate constant for hepatic elimination and K_e is the first order rate constant for urinary excretion. The rate of delivery to an organ such as muscle (m) is given by the blood flow to muscle (Q_m) times the arterial concentration (C_a). The rate of removal from the muscle is blood flow (Q_m) times the venous concentration (C_{vm}).

DETERMINANTS OF PHARMACOKINETICS

1. Absorption

Bioavailability

Bioavailability may be defined as the percentage or fraction (F) of administered drug that reaches systemic circulation in a chemically unchanged form. The bioavailability of any drug after intravenous administration is 100 per cent (F = 1) and is generally assumed to be closer to 100 per cent when given intramuscularly or subcutaneously. The bioavailability of drugs after oral administration ranges between 0 and 100 per cent depending on the extent of absorption and first-pass effects. Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. Drugs undergoing significant first-pass metabolism also show low bioavailability. In view of this, the bioavailability generally refers to oral bioavailability and less applicable to other routes. It is an absolute term.

Bioavailability (Absolute bioavailability) is determined by measuring the area under plasma drug concentration versus time curve (AUC) after oral or other non-vascular routes and comparing this with AUC measured after intravenous administration (100% bioavailability) of the same drug. If the AUC for both curves are equal, then bioavailability is 100 per cent (F = 1).

$$\text{Bioavailability (oral)} = \frac{AUC_{oral}}{AUC_{IV}} \times 100$$

Or

$$F = \frac{AUC_{oral}}{AUC_{IV}}$$

Bioavailability is a useful parameter that is used to predict drug efficacy after different routes of administration or administration of different formulations of the same drug. The influence of route of administration on drug bioavailability is generally in the given order: Parenteral route > oral route > topical route. Factors those determine absorption of a drug generally also determine its bioavailability.

Relative bioavailability: In contrast to absolute bioavailability (Oral vs IV availability of same drug product), when the systemic availability of a proprietary drug/formulation after oral administration is compared with that of an oral standard of the same drug, it is called relative bioavailability. It is denoted by symbol F_r.

Bioequivalence: Two related drugs are said to be bioequivalent if they show comparable bioavailability and similar times to achieve peak blood concentrations. Bioequivalence is also said to exist when the bioavailability of a drug from different formulations is the same. Bioequivalence, thus, indicates that different drug products, when given to the same patient in the same dosage regimen, result in equivalent concentrations of drug in plasma and tissues. Bioequivalent products are also expected to be therapeutically equivalent (same therapeutic and adverse effects). Two related drugs or different formulations of the same drug with a significant difference in bioavailability are said to be bioinequivalent.

Area under curve (AUC)

Area under curve (AUC) may be defined as the total integrated area under plasma drug concentration-time curve and expresses the total amount of drug that comes into the systemic circulation after administration of drug. It is an important parameter in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption. It is also used to calculate several other pharmacokinetic parameters. The AUC is expressed as the product of concentration of drug and time (μg/ml x hours).

The AUC can be calculated in several ways. The most common method is by dividing the Y intercept by the slope (rate constant) of the respective component. Therefore, for a one compartment model the AUC = B/β and for a two compartment model AUC = A/α + B/β, and so on. However, this method requires that the data of plasma drug concentration–time curve be 'fit' or forced into a model (e.g. one compartment or two compartments). If the model does not fit data very well, the pharmacokinetic parameters derived from the model do not accurately represent the data. An alternative method for calculating AUC is based on the trapezoidal method using non-compartmental analysis. This method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the area of each trapezoid and adding them together. The advantage of trapezoidal method is that data are not forced to fit a model, which may minimise error introduced by data manipulation.

2. Distribution

Volume of distribution

Volume of distribution/Apparent volume of distribution (V_d) may be defined as an apparent (hypothetical) volume of body fluid into which the total administered drug would have to be distributed (diluted) to produce the desired concentration in plasma. Here, the assumption is made that the body behaves as a single homogenous compartment with respect to the drug.

$$\text{Volume of distribution } (V_d) = \frac{\text{Amount of drug in body}}{\text{Plasma drug concentration}}$$

$$= \frac{\text{Dose (D)}}{C}$$

For example, if 500 mg of a drug is given to a patient and the subsequent plasma concentration is 10 mg/L, then 500 mg seems to be distributed in 50 L. Volume of distribution has nothing to do with the actual volume of the body or its fluid compartments but rather provides only an estimate of the extent of distribution of a drug within the body. Drug distribution in body compartments varies according to the physicochemical properties of the drug. Thus, V_d is a characteristic property of the drug rather than the patient, although disease states may influence V_d. It is expressed in litres.

The apparent volume of distribution for a drug may be determined by a number of methods including extrapolation and area methods.

Extrapolation method: It is the simplest but less accurate method for calculating volume of distribution. In this method, a known amount (dose) of a drug is given by single intravenous route (IV route is selected because it is the only route by which whole of administered drug reaches systemic circulation) and the maximum drug concentration is determined after distribution equilibrium has been reached, but before excretion has begun. This is obtained by extrapolating the linear terminal phase (elimination phase, β) of the plasma drug concentration-time curve to the Y-axis (see Figure 5.3). The value of B (or C_0) thus obtained is substituted in the above given equation:

$$V_{d(B)} = \frac{\text{Dose}}{B} \quad \text{or} \quad \frac{\text{Dose}}{C_0}$$

Where $V_{d(B)}$ is the volume of distribution (L/litre) calculated by extrapolation method, dose is the amount (mg or g), and B is the zero-time intercept of terminal elimination phase. Because the apparent volume of distribution can be used to calculate the amount of a drug needed to achieve a desired plasma concentration, it is common to divide the apparent volume of distribution by the animal's weight so that the units are mL/kg or L/kg.

Since the extrapolated method neglects the distribution phase of drug disposition, it is more suitable for calculating V_d for drugs which behave according to one compartment model. For drugs which follow two-compartment model, extrapolation method often gives an overestimate of the V_d value.

Area Method: The area under curve method provides a more satisfactory way of calculating volume of distribution parameter. It describes the disposition kinetics of drugs by a useful and reliable method that can be applied to many compartmental models for estimating V_d.

For drugs given as IV bolus

$$V_{d(area)} = \frac{\text{Dose}}{AUC.\beta}$$

For drugs given by extravascular routes

$$V_{d(area)} = \frac{\text{Dose} \,.\, F}{AUC.\beta}$$

Where $V_{d\,(area)}$ is the volume of distribution (L/litre) calculated by area under curve method, dose is the amount of drug (mg or g), β is the slope (rate constant) of elimination phase, and F is the fraction of drug absorbed into systemic circulation. The F is equal to one (i.e. complete bioavailability) when the drug is administered by intravenous route.

The value of V_d serves as proportionality constant relating the plasma concentration of a drug to the total amount of drug in body. A large apparent volume of distribution implies wide distribution or extensive tissue binding, or both. Lipid soluble drugs are usually widely distributed in body fluids and tissues and have large volumes of distribution, which may exceed the actual volume of the body i.e. 1 L/kg (therefore, volume of distribution is mentioned as apparent or hypothetical because fluid volume >1 litre/kg body weight never exists in the body). On the other hand, water soluble and ionised drugs tend to be confined to the circulation and have a low volume of distribution i.e. < 0.3 L/kg. The V_d of drugs those are highly protein bound (>80%) also tends to be small, reflecting their confinement of the extracellular fluid. The value of V_d is usually constant over a wide range for a given species of animal.

3. Elimination

Half-life (t½)

Half-life (t½) may be defined as the time taken for the concentration of a drug in plasma to decline by one-half or 50% of its initial value (or it may be defined as the time required for the body to eliminate half of the drug). This value is determined during the elimination phase of a drug; therefore it is also called elimination half-life. Occasionally the term plasma half-life is also used for the same purpose. The half-life is inversely related to the elimination rate constant β (i.e. faster the elimination, shorter the half-life) and is expressed in hours or minutes. Mathematically it is calculated as given below:

$$\text{Half-life } (t_{½}) = \frac{\ln 2}{\beta} = \frac{0.693}{\beta}$$

Where, ln 2 is the natural logarithm of 2 (or 0.693). Half-life of a drug can also be readily obtained from the β-slope of the plasma drug concentration-time profile as depicted in Figure 5.3. The elimination half-life can also obtained from clearance (Cl) and apparent volume of distribution (V_d) according to the following equation.

$$\text{Half-life } (t_{½}) = \frac{0.639 \,.\, V_d}{Cl}$$

The half-life is most widely used measure depicting the duration of action of drug and is closely related to the concentration of drug at site of action i.e. a short half-life indicates short duration (rapid elimination) and, therefore, low concentration at the site of action. Half-life is one of the most useful parameters for determining an appropriate dosing interval. Additionally, it determines the time taken to achieve a steady state or equilibrium constant with any constant dose rate. Half-life value of majority of drugs is independent of the dose administered because their overall elimination follows first-order kinetics (i.e. a constant fraction of drug present in plasma is eliminated in unit time). In such cases, complete elimination (> 95%) occurs in 5 half-lives [i.e., 50 % of drug is eliminated in one half-life, 75% (50 + 25) in two, 87.5% (50 + 25 + 12.5) in three, 93.75% (50 + 25 + 12.5 + 6.25) in four, and 96.88% (50 + 25 + 12.5 + 6.25 + 3.13) in five half-lives]. When the drug obeys zero-order kinetics (i.e. a constant amount of drug present in plasma is eliminated in unit time), the half-life of drug depends on the administered dose i.e. it becomes progressively longer as the dose is increased. The half-life of salicylates in cats and phenylbutazone in dogs and horses are dose-dependent (see for detail, orders of pharmacokinetic processes). The half-life of a drug is influenced by all factors which govern the elimination (e.g. plasma protein binding, type of metabolic reaction, metabolising enzyme induction or inhibition, blood flow to liver or kidneys, efficiency of renal excretion, disease states, drug interactions, etc.). In general, drugs which are widely distributed and sequestered in tissues have a long half-life. Entero-hepatic circulation and plasma protein binding also increase the half-life. In renal or hepatic dysfunctions, the half-life of a drug may increase, depending on the route of elimination.

Other types of half-life: Occasionally, the term half-life is used for purposes other than the elimination of drugs. These may include absorption half-life (time in which 50% of the administered drug is absorbed), distribution half-life (time in which 50% of the administered drug is distributed) and biological half-life (time in which 50% of the pharmacological effect produced by a drug is declined by one-half). These terms are less frequently used.

Clearance

Clearance (Cl)/ or Body clearance (Cl_B) may be defined as the theoretical volume of plasma cleared of the drug by various elimination processes per unit time. Body clearance is expressed in terms of volume per unit time (ml/min), and sometimes it is corrected for the body weight of animals (ml/kg/min). Body clearance relates plasma drug concentration with the rate of drug elimination according to the following equation:

$$\text{Body clearance} = \frac{\text{Rate of eliminaton}}{\text{Plasma drug concentration}}$$

Pharmacokinetically, body clearance is obtained as the product of apparent volume of distribution and overall elimination rate constant. Thus, if the V_d and β (or t½) of a drug are known, the body clearance of the drug can be determined:

$$\text{Body clearance } (Cl_B) = \beta . V_d = \frac{0.693 V_{d(area)}}{t_{1/2}}$$

The equation shows that half-life changes inversely (and proportionately) with the clearance of drug i.e. as the body clearance increases, the half-life (t½) of drug decreases. However, short half-life of a drug should not be always equated with high body clearance because the body clearance parameter comprises both the volume as well as the elimination rate constant. Change in volume of distribution, as in obesity or oedematous conditions, will also reflects change in body clearance.

Body clearance is considered to be a better index of efficiency of drug elimination than the commonly used half-life because it (body clearance) is independent of disposition kinetics of the drug (e.g. distribution). Unlike β and t½, which depend on K_{12}, K_{21} and K_{el}, the body clearance changes in exactly in proportion to K_{el}. However, body clearance is practically not a useful parameter in the design of dosing regimen because it expresses only the rate of drug removal from body and not how long a drug stays in body (clearance expresses the rate or efficiency of drug removal from plasma but not the amount of drug eliminated).

Organ clearance: Clearance at an individual organ level is called organ clearance. It is often used to assess the excretory capacity and, thus, physical well being of an organ. It can be estimated by dividing the rate of elimination by each organ with the concentration of drug presented to it.

$$\text{Renal clearance } (Cl_R) = \frac{\text{Rate of elimination by kidney}}{\text{Plasma drug concentration}}$$

$$\text{Hepatic clearance } (Cl_H) = \frac{\text{Rate of elimination by liver}}{\text{Plasma drug concentration}}$$

$$\text{Other organ clearance } (Cl_{Others}) = \frac{\text{Rate of elimination by other organs}}{\text{Plasma drug concentration}}$$

The total body clearance (Cl_B), sometimes called total systemic clearance, is an additive property of individual organ clearances.

$$Cl_B = Cl_R + Cl_H + Cl_{Others}$$

Clearance by all organs other than kidney is sometimes known as non-renal clearance (Cl_{NR}). It is the difference between total clearance and renal clearance.

$$Cl_B = Cl_R + Cl_{NR}$$

Or $$Cl_{NR} = Cl_B - Cl_R$$

If a drug is cleared exclusively by one organ, then total body clearance also represents clearance of the specific organ and can be used to evaluate the function of the organ.

Design of Dosage Regimen

Dosage regimen is defined as the manner in which a drug is administered. For some drugs such as antiemetics, pre-anaesthetics and anthelmintics, single dose of the drug may provide effective treatment. In such cases, there is no chance of drug accumulation and a constant therapeutic plasma concentration is not considered essential for treatment. However, for many drugs like antimicrobials, antihypertensives and antiarrhythmics, multiple doses of the drug at appropriate intervals over a period of time are required for successful therapy. In these cases, design of an optimal dosage regimen is necessary.

Irrespective of the route of administration, a dosage regimen is composed of two important variables: the magnitude of each dose (dose size) and the frequency with which the dose is repeated (dosing interval).

Dose

Dose or dose size is a quantitative term estimating the amount of drug, which must be administered to produce a particular biological response i.e. to achieve a specified target plasma drug concentration. As the magnitude of both therapeutic and toxic responses depends on plasma drug concentration, size of dose should be so selected that it produces the peak plasma concentration (C_{max}) or steady state level within the limits of therapeutic range (between minimum effective concentration and maximum safe concentration). The therapeutic plasma concentration range is obtained by careful clinical evaluation of the response in a sufficient number of appropriately selected individuals (for antimicrobials, the range is based upon the minimum inhibitory concentration for susceptible microorganisms).

The dose (D) necessary to achieve peak or maximum therapeutic concentration (C_{max}) depends on the volume of distribution (V_d), and is calculated by the following equation:

$$D = C_{max} . V_{d\,(area)}$$

When the drug is administered by other than intravenous route, its bioavailability (F) may be less than 1.0 and must be taken into account.

$$D = C_{max} . V_{d\,(area)}/F$$

Since size of dose for drugs which do not accumulate depends on the apparent volume of distribution, the dose of drug must be increased or decreased proportionately with changes in V_d in order to establish the required plasma drug concentration.

Dosing interval

Dosing interval is the time interval between doses. It ensures maintenance of plasma concentration of a drug within the therapeutic range for entire duration of therapy. Dosing interval should be selected primarily by consideration of the fluctuations in drug concentration (ratio C_{max}/C_{min}) that can be tolerated without excessive toxicity or loss of efficacy and the overall elimination rate constant (β).

The dosing interval is determined by the time (T_{max}) it takes for maximum plasma concentration (C_{max}) to drop to a point below which the desired response no longer occurs (MEC, C_{min}).

$$T_{max} = \frac{\ln [C_{max} / C_{min}]}{\beta}$$

Where, ln is natural logarithm. On the basis of above equation, if C_{min} for a drug is equal to the half of C_{max} (i.e. $C_{max}/C_{min} = 2$), the dosing interval is equal to the half-life of the drug.

$$T_{max} = \frac{\ln [C_{max} / C_{min}]}{\beta} = \frac{\ln (2)}{\beta} = t_{1/2}$$

Accordingly for most drugs, a dosing interval equal to or less than the elimination half-life is recommended for therapeutic

purposes. For a given fluctuation (ratio C_{max}/C_{min}), the smaller the value of β (i.e. longer the half-life), the longer will be the dosing interval. Therefore, dosing interval is related primarily to the half-life of drug.

Steady-state concentration (C_{ss})

Steady-state (ss) is defined as the time during which concentration remains stable or consistent when drug is given repeatedly or continuously (IV infusion). The time to reach steady state is a function of elimination half-life (t½) and is achieved when the rate of drug entering systemic circulation equals the rate of elimination. For most drugs, the steady state concentration is reached in approximately 5 half-lives. The time to reach steady state is independent of dose size, dosing interval and number of doses.

Multiple dosing

When a drug is administered in a fixed dose at fixed intervals, the plasma concentration rises exponentially to a plateau or steady state with a half time of increase that is equal to the elimination half-life of the drug. Thus 50% of the steady state level is achieved in one elimination half time, 75% (50 + 25) in two, 87.5% (50 + 25 + 12.5) in three, 93.75% (50 + 25 + 12.5 + 6.25) in four, 96.88% (50 + 25 + 12.5 + 6.25 + 3.13) in five, and so forth [when the first dose D_0 is administered at time 0, the amount of drug in body will be $D = D_0$. At the next dosing interval (equal to t½) when $D = ½ D_0$, the amount remaining in the body, administration of next dose (D_0) raises body concentration to $D = D_0 + ½ D_0$, and so forth]. During the initial period, the intake is more than the elimination, so there is continuous rise in plasma concentration. Subsequently, when the intake equals the elimination, a steady state is reached. In practice, a useful estimate of time to reach a steady state is obtained by the following equation:

Time to 95% steady state = $4.3 \times t_{½}$

Therefore, shorter the half-life, the more rapidly steady state is reached, and vice-versa. For drugs those follow first order kinetics, average (mean) steady state plasma concentration C_{ss}^{-} can be predicted from the following equation:

$$C_{ss}^{-} = \frac{F.D}{Cl_B.T}$$

Where, F is the fraction of the dose absorbed, D is the dose, Cl_B is the total body clearance and T is the dosing interval.

When a drug is given repeatedly, the plasma drug concentration rises to a maximum or peak (C_{max}) and then falls to a minimum or trough (C_{min}) during each dosing interval. During steady state level, the entire cycle is repeated identically in each interval (Figure 5.6). Generally during steady state, $C_{ss, min}$ (minimum concentration at steady state) equals the amount of drug in body after the first dose ($C_{1\ max}$), and the $C_{ss, max}$ (maximum concentration at steady state) equals twice the first dose ($2 \times C_{1\ max}$). Therefore at steady state, $C_{ss, max} - C_{ss, min} = C_{1\ max}$, and $C_{ss, max}/C_{ss, min} = 2$. All this applies when dosing interval (T) is equal to elimination half-life (t½).

Accumulation

Accumulation is the ratio of maximum plasma concentration following the dose at steady state compared with that following the first dose (i.e. $C_{ss, max}/C_{1\ max}$). The extent to which a drug accumulates in body during multiple dosing is a function of dosing interval and elimination half-life, but is independent of dose size. If the elimination half-life is equal to the dosing interval ($T = t½$) then at steady state level, the drug will accumulate two fold ($C_{ss, max}/C_{1\ max} = 2$). When $T < t½$, the degree of accumulation will be greater, and drug may show toxic responses.

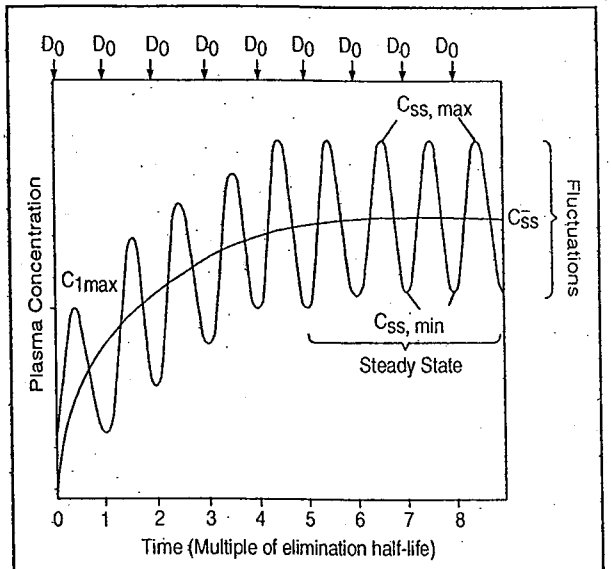


Fig. 5.6. Schematic representation of a plot of plasma-drug concentration versus time following repeated dosage at fixed intervals of a drug (See text for details).
Abbreviation : D_0=dose, C_{1max}=maximum concentration after first dose ; $C_{ss, min}$ = minimum concentration at steady state; $C_{ss, max}$ = maximum concentration at steady state ; C_{ss} = mean plasma concentration at steady state.

Fluctuation

Fluctuation is defined as the ratio of the maximum concentration to the minimum concentration of drug in plasma at steady state (i.e. C_{max}/C_{min}). As a general rule, greater the ratio, greater will be the fluctuation. Fluctuation primarily depends on dosing interval and half-life of drug, but is also affected by dose size. For a given dose, if dosing interval is increased, C_{max} and C_{min} will decrease (drug efficacy will decrease), but the fluctuations will increase. The opposite is observed when dosing interval is reduced; toxicity of drug in such cases may increase due to greater drug accumulation (Figure 5.7B). On the other hand, for a given dose interval, if dose size is increased, C_{max} and C_{min} will increase with greater fluctuations during each dosing interval; toxicity of drug in such cases also increase due to higher drug concentrations (Figure 5.7A). The greatest fluctuation is observed when the drug is given as IV bolus. Fluctuations are small when the drug is given extravascularly because of continuous absorption of the drug. Administration of smaller doses in shorter dose intervals results in smaller fluctuations. Administration of drugs by intravenous infusion maintains a constant steady state or plateau level without fluctuations (IV infusion is the only method which does not produce fluctuations).

Clinical application

Clinically, a proper balance between both dose size and dosing frequency is often desired to attain steady state concentration with minimum fluctuations and to ensure therapeutic efficacy and safety. In therapeutics, choice of dosage interval usually represents

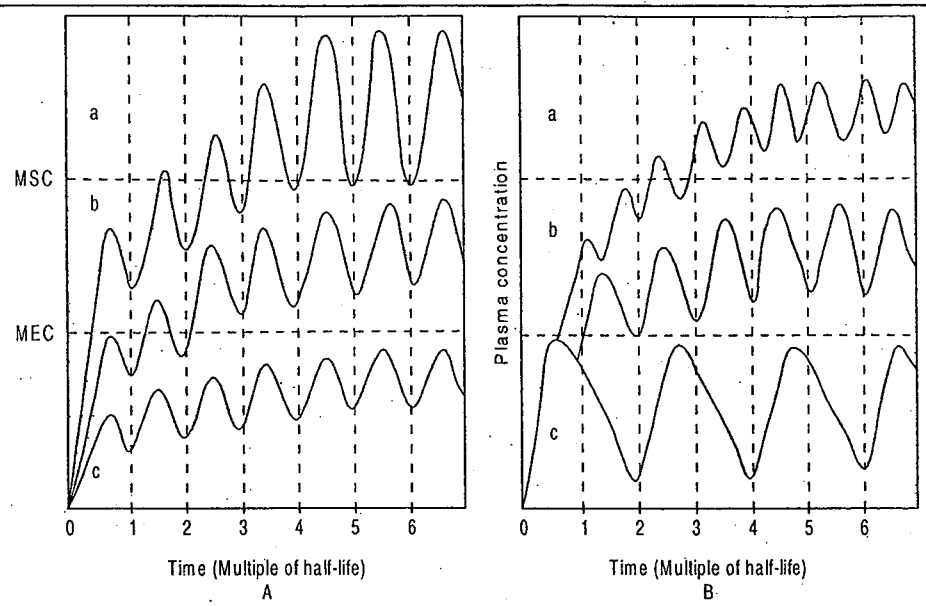


Fig. 5.7. Schematic representation of plots of plasma drug concentration versus time following repeated administration of a drug.
(A) : Influence of dose sizes at fixed dosing inteval.
a. Large dose size : Larger fluctuations with both therapeutic and toxic responses (Dose is pharmacologically unsuitable).
b. Optimum dose size : Moderate fluctuations (within therapeutic range) with therapeutic responses (Dose is pharmacologically suitable).
c. Small dose size : Smaller fluctuations, but it shows sub-therapeutic responses (Dose is pharmacologically ineffective).
(B) : Influence of dosing intervals at fixed dose.
a. Shorter dosing interval ($T < t_{1/2}$) : Smaller fluctuations but it shows both therapeutic and toxic responses (Dosing interval is pharmacologically unsuitable).
b. Optimum dosing interval ($T = t_{1/2}$) : Moderate fluctuations (within therapeutic range) with therapeutic responses (Dosing interval is pharmacologically suitable).
c. Larger dosing interval ($T > t_{1/2}$) : Larger fluctuations with sub-therapeutic response (Dosing interval is pharmacologically ineffective).
Abbreviations : MEC = minimum effective concentration; MSC = maximum safe concentration.

a compromise between the need for minimising the extent of fluctuations in steady state concentration (based on therapeutic range) and the inconvenience of frequent dosing. In veterinary medicine, a dosage interval of twice the half-life might be considered suitable for most antimicrobials, analgesics, anticonvulsants, etc. To engender owner compliance with drug administration, a dosage interval of 4, 6, 8, 12 or 24 hours, depending on the duration of action of drug, is normally recommended.

Administration of priming or loading dose, usually parenterally, followed by a series of maintenance dose at regular intervals help to produce an immediate and sustained effect. The priming dose allows one to achieve plasma drug concentrations above the minimum effective concentration (MEC) quickly. The use of priming dose to attain target concentration rapidly depends on the urgency of treatment and the half-life of drug. For drugs which have a long half-life (e.g. digoxin) and for antimicrobial drugs, the desired plasma drug concentration may be achieved more rapidly by administration of a priming dose. For drugs with a narrow margin of safety like digoxin, the priming dose is given in small fractions at short intervals, usually less than the t½ of the drug. This obtains an early steady state level with least fluctuations which is similar to that observed with constant rate infusion or controlled release system. The priming dose may be calculated from the equation:

$$D_l = (V_d \times C_{ss})/F$$

where D_l = priming dose, C_{ss} = steady-state concentration, V_d = volume of distribution and F = bioavailability. When the loading dose is administered parenterally (intravenous), F can be considered to be equal to 1.

Intravenous infusion

For constant IV infusions, zero order absorption and first order elimination apply. When a drug is infused at a constant rate (zero-order input) and the disposition (distribution and elimination) follows first-order kinetics, the drug level will accumulate until a plateau or steady state concentration is reached. When the plateau level is attained, the steady state plasma drug concentration (C_{ss}) is given by the expression:

$$C_{ss} = \frac{R_0}{\beta \,.\, V_{d(area)}} = \frac{\text{Rate of infusion}}{\text{Body clearance}}$$

Where, R_0 is the rate of infusion, β is the overall elimination rate constant, and $V_{d(area)}$ is apparent volume of distribution. Since the kinetic constants (β and V_d) are fixed values for a drug, the steady state or plateau concentration of the drug is directly related to the infusion rate. Increasing or decreasing the infusion rate leads to increasing or decreasing the plasma concentration at plateau, respectively, but does not influence the rate at which plateau is attained. Similar to multiple dosing, the rate of approach to the plateau value will always be same and determined solely by the elimination rate constant or the half-life of drug. When the infusion is terminated, the plasma drug concentration will decline in exponential fashion.

Intravenous infusion is mostly used for drugs those have a narrow range of therapeutic plasma levels (e.g. antiarrhythmic agents) because it is the only method, which maintains a constant steady state or plateau level without fluctuations (plasma drug concentrations fluctuates within 5 per cent of the plateau level). Drugs having very short half-lives (e.g. dopamine) are solely administered by IV infusion.

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CHAPTER 6 PHARMACODYNAMICS

Mechanisms of drug action
- Specific action
- Non-specific action

Drug receptors
- Types of receptors
  - Ionotropic-receptors/Ligand gated ion-channels
  - G-protein coupled receptors
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  - Receptors regulating DNA transcription

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- Types of drug
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- Change in number of receptors
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Dose-response relationship
- Graded or Gradual dose-response relationship
- Quantal or All-or-none dose response relationship

Variables of dose-response curve
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Measures of safety
- Therapeutic index
- Therapeutic ratio
- Certain safety factor
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- Mechanisms of drug interaction
- Types of drug interaction
  - Addition
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  - Synergism
  - Antagonism

Antagonism
- Chemical antagonism
- Functional antagonism
- Disposional antagonism
- Receptor antagonism

Pharmacodynamics may be defined as the study of biochemical and physiological effects of drugs and their mechanisms of action. This aspect of pharmacology deals with the action of drugs within body and, therefore, is concerned mainly with the interaction of drugs with their target sites. It is also concerned with the correlation between pharmacological activity and chemical structure of the drug.

MECHANISMS OF DRUG ACTION

Mechanism of action may be defined as the means by which a drug produces its pharmacological effect. During its action, a drug cannot create or impart a new function to a cell or tissue but it only modulates the rate at which an inherently possessed biological function proceeds. For example, drugs can speed up or slow down the biochemical reactions which cause heart to contract, endocrine glands to secrete hormones, blood to clot and nerves to propagate impulses. The pharmacological effects of most drugs result from their interaction with some macromolecular component of the organism. These interactions often alter the function of the pertinent component and thereby initiate some biochemical and physiological changes which are characteristic of the response of the drug. In some cases, this interaction is highly specific whereas in others it is non-specific.

I. SPECIFIC ACTION

Specific actions are produced by drugs, which interact with certain specific macromolecular components in the body. Drug interaction with these macromolecules shows structure activity relationship (SAR) and can be blocked by specific antagonists. These macromolecules include receptors, ion-channels, enzymes and carriers.

1. Receptors

A receptor is a specific macromolecular component usually made up of a protein or glycoprotein present on the membrane surface or within the cell to which a specific hormone, neurotransmitter or drug combines to produce its physiological or pharmacological action. Receptor that affects the activity of a cell

directly by acting on an ion channel is called the ionotropic receptor and that indirectly by first initiating a metabolic change in the cell is called the metabotropic receptor. Both ionotropic and metabotropic receptors are the most important targets of drug action in the body. Activation of receptors by drugs leads to initiation of pharmacological effects, whereas inhibition of receptors by blockers terminates pharmacological effects. Some ion-channels, enzymes and carrier-mediated molecules can also be activated or inhibited indirectly by receptor-mediated actions. Examples of drugs mediating their action through receptors are epinephrine, atropine, morphine and diazepam.

2. Ion-channels

An ion-channel is a space or pathway through the cell membrane, which allows free movement of a specific ion like sodium, potassium, calcium or chloride. The ion-channels are broadly of two types: voltage gated channels and ligand gated channels. The movement of ions through an ion channel alters the ionic concentration and thus helps to establish a voltage gradient across the plasma membrane of the cells. In neuronal cells, the resultant shifts in membrane potential are either depolarization or hyperpolarization, making the cell more or less excitable, respectively. The voltage gated ion channels are modulated mainly directly, whereas the ligand ion channels can be modulated by drugs through receptors. Direct modulation of voltage-gated ion channels occurs by binding of the drug mainly directly to some parts of the channel protein. In this case, the process of channel opening may be either inhibited or facilitated by the drug. Examples of drugs directly modulating ion channels include calcium channel blockers (e.g. verapamil and diltiazem), sodium channel blockers (e.g. procainamide) and chloride channel openers (e.g. diazepam). Modulation of ligand-gated ion-channels occurs only when a drug interacts with its specific receptor (as discussed above). In this case, the ion-channels are either linked directly to a receptor (ionotropic receptor) or indirectly through a G-protein and other intermediaries (metabotropic receptor). Ion-channel modulation by drugs, acting directly or indirectly, is one of the most important mechanisms by which pharmacological effects are produced at the cellular level.

Voltage gated ion-channels: Voltage gated sodium, calcium, potassium and chloride channels are essential for a wide range of physiological functions such as neurotransmitter secretion, cardiac impulse generation and muscle contraction. Several drugs acting on voltage gated-sodium, calcium and potassium channels have been synthesised in recent years, but the pharmacology of voltage gated chloride channels is not yet developed.

a. ***Voltage-gated sodium channels:*** Voltage-gated sodium channels (Na_V channels) play a critical role in initiating the action potential. The activation of sodium channel allows for the inward movement of Na^+ from the extracellular space of the cell. Several groups of drugs such as local anaesthetics (e.g. lidocaine and procaine), antiarrhythmics (e.g. procainamide) and anti-epileptics (e.g. phenytoin, felbamate and carbamazepine) are known to mediate their action through inhibition of voltage-gated sodium channels. Some natural toxins (e.g. tetrodotoxin and saxitoxin) and synthetic toxicants (e.g. pyrethroid and organochlorine insecticides) also mediate their effect through blockage of Na_V channels.

b. ***Voltage-gated calcium channels:*** Three types of voltage-gated calcium channels (Ca_V channels) have been identified viz. Ca_{V1}, Ca_{V2} and Ca_{V3}. The Ca_{V1} located in skeletal muscle, bone (osteoblasts), ventricular myocytes and dendrites mediates L-type Ca^{2+} currents and are targets of antiarrhthymic (e.g. verapamil) and antihypertensive (e.g. nifedipine) drugs. The Ca_{V2} present in neuronal tissues mediates P/Q type, N-type (mainly) and R-type Ca^{2+} currents and control the release of neurotransmitters at the presynaptic level. Ziconotide, a synthetic peptide analogue of an ω-conotoxin peptide and a NSAID approved for the intrathecal treatment of severe chronic pain, selectively block the N-type Ca_V. The Ca_{v3} channels present in neurons and cardiac cells which have pacemaker activity mediates T-type currents. Selective inhibition of T-channels may have clinical importance in cardiovascular diseases, some forms of epilepsy, sleep disorders, pain and possibly cancer.

c. ***Voltage-gated K^+ channels:*** Voltage-gated K^+ channels (K_V channels) are transmembrane channels specific for potassium ion. During action potentials, they play a crucial role in returning the depolarized cell to a resting state. Various types of voltage-gated potassium channels have been identified and include the slow delayed rectifiers $K_{V(s)}$ and the rapid delayed rectifiers $K_{V(f)}$. Voltage and G-protein-gated K^+ channels, voltage and calcium-activated K^+ channels, and ATP-dependent K^+ channels (K_{ATP}) also exist. Sulphonylureas (e.g. tolbutamide and glibenclamide) and glinides (e.g. repaglinide) block the pancreatic β-cell K_{ATP} channels, whereas vasodilators such as cromakalim, pinacidil and diazoxide directly activate and open the K_{ATP} channels.

3. Enzymes

An enzyme is a protein that catalyzes a chemical reaction by lowering its activation energy. Some enzymes are highly selective for their substrates and speed up only a few reactions, while others are non-selective and catalyze many chemical reactions. In the biological system, the enzyme activity can be inhibited by other molecules including several drugs and poisons. Some drugs interact only with specific enzymes and inhibit their selective action while others can influence the non-specific enzymes with variable actions. In most cases, drugs behave as a substrate analogue that acts as a selective inhibitor of some specific enzyme either reversibly (e.g. physostigmine acting on cholinesterase enzyme) or irreversibly (e.g. aspirin acting on cyclooxygenase enzyme). Selective inhibitors for various cyclic nucleotide phosphodiesterases (e.g. sildenafil for PDE-5) and selective cyclooxygenase inhibitors (e.g. celecoxib for COX-2) have been developed. In other cases, drugs act like a false substrate and undergo some chemical transformation by enzymes to form an abnormal product(s), which subverts the normal metabolic pathways. For example, antihypertensive drug α-methyldopa mimics the norepinephrine precursor dopa, causing norepinephrine to be partly replaced by pseudotransmitter α-norepinephrine, thus affecting function of the sympathetic nervous system.

4. Carriers

A carrier (Transporter) is generally a membrane protein that transports specific ions and small organic molecules across the cell membranes. Each carrier protein is designed to recognize only one

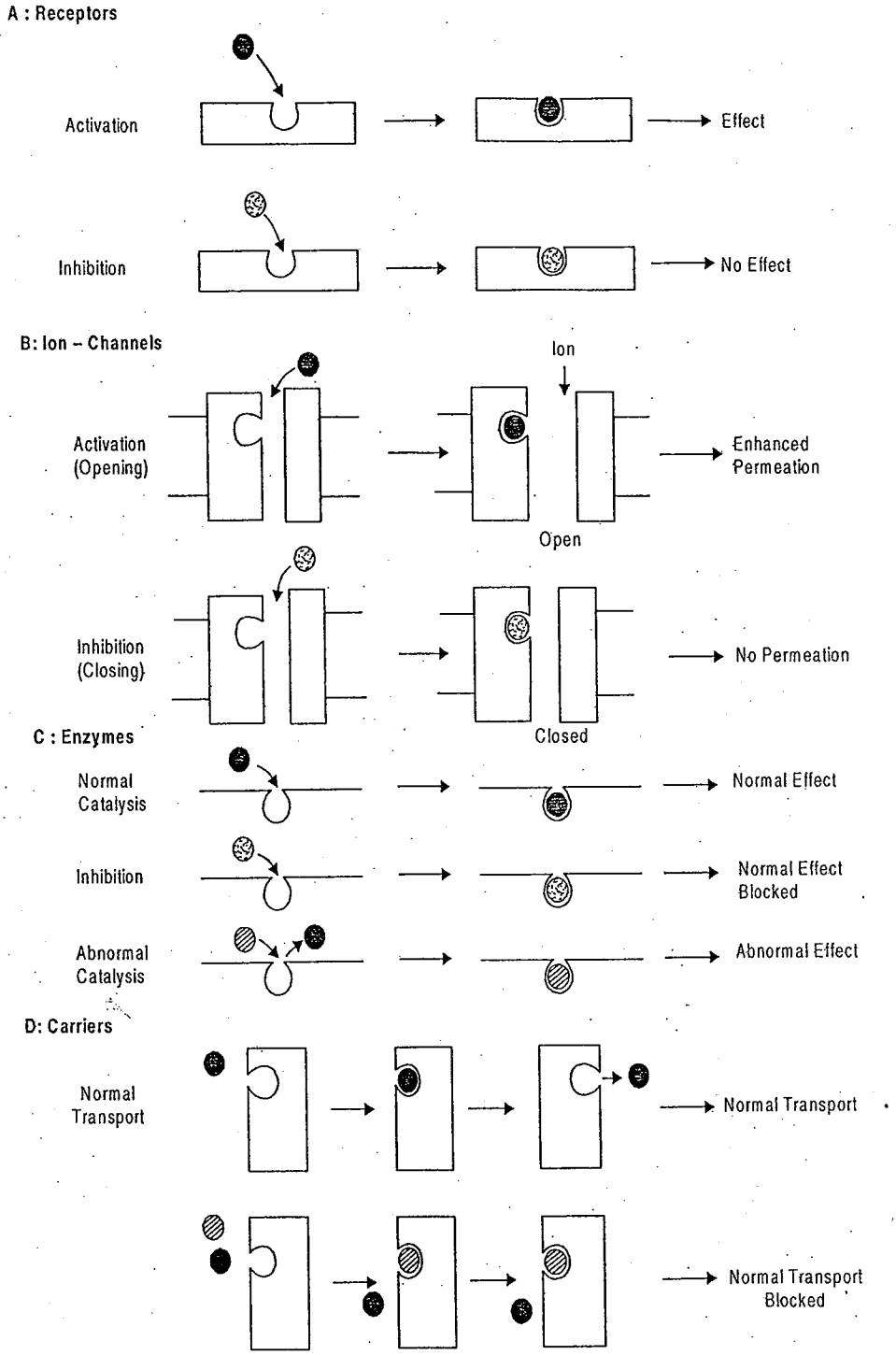


Fig. 6.1. Schematic representation of specific actions of drugs (See text for detail).
● = activator/agonist/normal substrate; ⊘ = inhibitor/antagonist; ▨ = false substrate

substance or one group of very similar substances, so is highly selective in the type or types of ions or molecules which are allowed to cross the membrane. The molecule or ion to be transported must first bind at a binding site at the carrier molecule on one side of the membrane and is then transported by the carrier to the other side of the membrane (See Figure 6.1). The carrier molecules are targets for several drugs those interact with specific recognition sites on the macromolecule and block the transport system. This occurs, for example, with antidepressant drugs (e.g. imipramine) which block reuptake of norepinephrine into adrenergic presynaptic nerve terminals and probenecid that blocks carrier meant for excretion of penicillins. Similarly, hemicholinium blocks choline carrier in the post-synaptic cholinergic nerve fibres. Other examples of drugs mediating pharmacological effects through inhibition of membrane transporters include cardiac glycosides (sodium pump, Na^+/K^+-ATPase), omeprazole and analogues (proton pump, H^+/K^+-ATPase), diuretics (thiazides for Na^+/Cl^- co-transporter and furosemide for $Na^+/K^+/Cl^-$ co-transporter), amfetamines and reserpine (vesicular monoamine

transporter, VMAT), antidepressant paroxetine (serotonin transporter, SERT), and artemisinin and derivatives (plasmodial sarcoplasmic and endoplasmic calcium ATPase, SERCA).

II. NON-SPECIFIC ACTION

A large number of drugs do not act by virtue of combination with functional cellular components or receptors. Their action can be explained purely on the basis of their physical or chemical properties. Unlike drugs having specific actions, these drugs do not react with specific macromolecules, tend not to show structure-activity relationships, and do not have specific antagonists.

1. Physical actions

Certain drugs act exclusively by physical means outside of cells. These sites include external surfaces of skin, respiratory tract and gastrointestinal tract. The action of these drugs is entirely due to possession of a common physical property unrelated to any one pertinent chemical structure and is therefore structurally non-specific. In contrast to the structurally specific compounds, many chemically unrelated compounds are capable of producing identical pharmacological action and their action is largely a function of the concentration rather than their interaction with some specific macromolecular component. For example, the bactericidal activity of cationic detergents is structurally non-specific and purely a function of their surfactant property. The pharmacological action produced by bulk and saline purgatives (e.g. bran and magnesium sulfate), osmotic diuretics (e.g. mannitol), adsorbents (e.g. activated charcoal), and protectives (e.g. talc) are also produced entirely due their physical properties. In a similar way, general anaesthesia produced by inhalant anaesthetics like diethyl ether, halothane and chloroform can be correlated well with the physical properties of their oil: water partition coefficients.

2. Chemical actions

The pharmacological actions produced by certain drugs are entirely due to their chemical properties. These drugs react extracellularly with some endogenous substances according to simple chemical reactions and thereby produce desirable therapeutic responses. Their action is, therefore, structurally non-specific and not related to binding to any functional macromolecular component of the cell. For example, antacids (e.g. aluminium hydroxide) neutralises gastric acid by a simple acid-base chemical reaction. In similar chemical reactions, acidifying (e.g. ammonium chloride) and alkalinising (e.g. sodium bicarbonate) agents react with buffers in plasma and alter the pH of urine. Germicidal actions of oxidising (e.g. potassium permanganate) and reducing (e.g. formaldehyde) agents, and chelating action of some antidotes (e.g. penicillamine and dimercaprol) are also produced by chemical actions.

DRUG RECEPTORS

The drug receptor is a macromolecular component of the body tissue with which a drug interacts to initiate its pharmacological effects. The existence of receptors was inferred from the experimental observations of John Newport Langley and Paul Ehrlich at the end of nineteenth century who suggested interaction of receptor with drug like a lock and its matching key (See for detail drug-receptor interactions). Receptors present in different cellular constituents are specific in size, shape and structure and allow interaction with specific ligands (physiological substances or drugs). Majority of drug receptors are cellular proteins whose normal functions are to mediate the action of endogenous regulatory ligands such as hormones, neurotransmitters, autacoids, etc. Nucleic acids and some other cellular constituents also comprise a category of drug receptors in the widest sense.

Signal transduction mechanism

The signal transduction is a mechanism that converts a chemical stimulus to a cell into a specific cellular response. Signal transduction starts when a ligand binds to a receptor and ends with a biological change in the cell. The entire process may take milliseconds (for ionotropic receptors), seconds or minutes (for G-protein- and enzymatic receptors) or hours and days (for steroid receptors). In the signal transduction mechanism, receptors can be regarded as the sensing elements and the cellular target bringing the biological changes as the effector. When a ligand binds to the ligand binding domain of a receptor, the shape or conformation of the receptor changes and it conveys the signal inside the cell. This is followed by a regulatory action that is exerted either directly on the effector or conveyed to the effector through an intermediary cellular molecule(s) called transducer or coupler. The receptor, its cellular target effector, and any intermediary molecule(s) are referred to as a receptor-effector system or signal transduction pathway. Sometimes, the effector protein may not be the ultimate cellular target in the chemical communication system. In such situations, the system may synthesise or release another signalling molecule, usually a small metabolite or ion, known as second messenger to carry forward the message of ligand (first messenger) to the ultimate cellular target. The important second messengers involved in signal transduction mechanism in most cells include cyclic adenosine monophosphate (cyclic AMP, cAMP), cyclic guanosine monophosphate (cyclic GMP, cGMP), inositol-triphosphate (IP_3), diacylglycerol (DAG), Ca^{++} and protein kinase C. Occasionally there is a long cascade of signals, one after the other ultimately leading to cellular response. With each step of the cascade, the signal can be amplified so a small signal can result in a large response. Receptors typically respond to only the binding of a specific ligand, which could be a neurotransmitter, hormone, growth factor, autacoid, drug or toxicant.

TYPES OF RECEPTOR

Drug receptors have been classified into four superfamilies according to their molecular structure and nature of signal transduction mechanisms. These superfamilies of receptor are ionotropic-receptors, G-protein coupled receptors, enzymatic receptors, and receptors regulating DNA transcription. These drug receptors are located either on the cell surface or inside the cell. Cell-surface receptors are mostly the integral membrane proteins those span the plasma membrane of the cell, with one part of the receptor on the outside of cell (the extracellular domain) and the other on the inside of cell (the intracellular domain). Signal transduction occurs as a result of the binding of a ligand to the extracellular domain of the receptor; the ligand itself does not pass through the plasma membrane. In contrast to cell surface receptors, the intracellular receptors are located entirely within the cells, either in the cytoplasm or inside the nucleus, and do not span the cell membrane. The chemical messenger itself has to pass through the cell membrane and bind directly to receptor in the cytoplasm or nucleus.

I. Ionotropic-Receptors/Ligand Gated Ion-Channels

Ionotropic-receptors (Ligand-gated ion-channels, iono-receptors or ion channel-linked receptors) are cell surface receptors those enclose ion selective channels (for Na^+, K^+, Ca^{++} or Cl^-) which are opened or closed in response to the binding of a chemical

messenger. The action of ionotropic receptors is similar to that of voltage-gated ion-channels, but they open and close in response to a ligand and not to the membrane potential. The direct link between ligand binding and opening or closing of the ion channel is also in contrast with the indirect function of metabotropic receptors, which use some intermediary substance such as G-protein or second messengers to open the channel. The ionotropic receptors are located primarily at nerve synapses and convert the chemical signal of presynaptically released neurotransmitter very quickly into a postsynaptic electrical signal with time lag between ligand binding and cellular response in only milliseconds. Examples of ionotropic-receptors include nicotinic-cholinergic receptor, $GABA_A$ receptor, glycine receptor, NMDA-receptor and $5\text{-}HT_3$ receptor.

Molecular structure

All ligand-gated ion-channels are oligomers, mostly pentamers, consisting of non-identical subunit proteins. The nicotinic-cholinergic receptor, a well characterized ionotropic-receptor with a molecular mass of 290 kDa, contains 5 subunits: two alpha subunits and one each of beta, delta and gamma subunits. The receptor subunits penetrate all the way through the membrane lying side by side in a circle to form a tubular channel (similar to petals of a lily). All subunits contain large hydrophilic intra- and extra-cellular segments and generally four hydrophobic membrane spanning domains. The long extracellular amino-terminal region of the subunits has, in common with many membrane proteins, sugar residues coupled to particular amino acids (glucosylation). It is followed by four hydrophobic regions which span the membrane, leaving the small carboxyl-terminus again on the extracellular surface (Figure 6.6). The two α subunits, containing one acetylcholine binding site each, constitute the ligand binding domain and function only when two molecules of acetylcholine bind simultaneously to the binding sites.

Similar to nicotinic receptors, the $GABA_A$ receptor, glycine receptor and $5\text{-}HT_3$ ionotropic receptors are made up of five subunits (pentamers). In contrast to these, the NMDA glutamate receptor contains four subunits (tetramer) and P2X-ATP receptor contains three subunits (trimers). Certain ionotropic-receptors also have secondary ligands, which bind to an allosteric site and modulate the gating of channel by the primary ligand (e.g. benzodiazepine receptor modulates $GABA_A$ gated Cl^- channels).

The ionotropic receptor can exist in three different conformational forms. The unoccupied-closed state is the protein at its original conformation. After the ligand binds to the binding sites on α subunits, the shape and conformation of the receptor changes and the receptor comes in an opened state, allowing extracellular ions to go into the cell. After a brief duration of milliseconds, the gate of receptor is closed again forming the third ligand occupied-closed state. The ligand then quickly dissociates from the receptor sites and the receptor returns to its original unoccupied-closed state and is ready for next transduction cycle.

Signalling mechanism

Ionotropic-receptors form ligand-regulated ion-selective channels in plasma membranes and do not involve any transducer or second messenger. Therefore, they convey their signal directly. When the ligand binds to the specific receptor ion-channel, conformational changes open the channel and the relevant one or more ions like Na^+, K^+, Ca^{2+} or Cl^- flow along the concentration gradient. This alters electric potential across the membrane, thereby causing either depolarisation (e.g. Na^+ channel and Ca^{++} channel) or hyperpolarisation (e.g. Cl^- channel and K^+ channel) of the cell. The ligand binding and channel opening occur on a millisecond time scale and the action usually decays within a few milliseconds. The fastest signalling system and drug action of the ionotropic-receptors are contributed mainly to the molecular structure of the receptor and direct coupling between the receptor and the ionic-channel.

II. G-Protein Coupled Receptors

G-Protein coupled receptors (GPCRs), also known as seven-transmembrane domain receptors (7TM receptors), serpentine receptor or G protein-linked receptors (GPLR), comprise a large superfamily of membrane bound receptors which regulate distinct effector proteins (enzymes, channels or carriers) through mediation of transducers, a group of guanine nucleotides (GTP and GDP) binding proteins known as G-proteins. The ligands those bind and activate these receptors include hormones, pheromones, neurotransmitters, autacoids and xenobiotics, which vary in size from small molecules to large proteins. The G protein-coupled receptors are involved in many diseases such as diabetes, blindness, allergies, depression, cardiovascular defects, and certain types of cancer, and presently are the target of approximately 30% of all therapeutic agents. Examples of G-protein coupled receptors include muscarinic-cholinergic receptors, histamine receptors, adrenergic receptors, eicosanoid receptors, 5-HT (except $5\text{-}HT_3$) receptors and many peptide hormones receptors. G-Protein coupled receptors are found only in eukaryotes.

Molecular structure

All G-protein coupled receptors have a common pattern of structural organisation. Most of the receptors consist of a single polypeptide chain of 400 to 500 residues. The polypeptide chain comprises of the extracellular amino-terminus followed by a long amino acid sequence having 7 α-helical membrane spanning hydrophobic regions with 3 extracellular and 3 intracellular loops (Figure 6.6) and then the intracellularly located carboxy-terminus. Both the extracellular amino-terminus and the intracellular carboxy-terminus vary greatly in length and sequence; the third intracellular loop is also a highly variable region. The agonist-binding site is located somewhere between the helices on the extracellular face, and not on extracellular N-terminal region as with the ionotropic-receptors. However, a substantial extracellular domain is important for the binding of negatively charge ligands like glutamate or the peptide hormones. A recognition site for the coupling G-protein is formed by a large intracellular loop.

The G-proteins are heterotrimeric molecules consisting of three subunits – α, β and γ attached to the inner surface of plasma membrane. They have highly a homologous guanine nucleotide binding domain, but distinct domains for interactions with receptors and effectors. There exist several types of G-proteins grouped into 4 families viz. G_s, G_i/G_o, G_q and $G_{12/13}$ and at least 20 different alpha (G_α) subunits based on their sequence homologies. These G-proteins can interact with different receptors and control different effectors (Table 6.1). The β and γ subunits are closely bound to one another and are referred to as the beta-gamma complex.

Table 6.1: List of important types of G-proteins distinguished by their α-subunits.

Families of G-protein	Alpha-subunit	Action on effector	Important cellular effect	Example(s)
G_s	α_s	↑ Adenylate cyclase ↑ Ca^{++} Channel	Increase heart rate, stimulate neuronal activity	Adrenergic β-R Serotonin 5-HT_4-R Dopamine-D_1 -R Histamine H_2-R
G_i/ G_o	α_i, α_o	↑ Adenylate cyclase ↑ K^+ Channel (via βγ subunits) ↓ Ca^{++} Channel	Smooth muscle relaxation, depress neuronal activity	Muscarinic M_2-R Adrenergic α_2-R Serotonin 5-HT_1-R Dopamine D_2-R Prostaglandin EP_1, EP_3, FP, & TP –R Opioid δ, κ, μ-R
$G_{q/11}$	α_q, α_{11}, α_{14}, α_{15}, α_{16}	↑ Phospholipase C	Smooth muscle contraction, Ca^{2+} flux	Adrenergic α_1-R Histamine H_1 Serotonin 5-HT_2-R Muscarinic M_1 and M_3 Metabotropic glutamate-R
$G_{12/13}$	α_{12}, α_{13}	↑ Rho family of GTPases ↑ Na^+/H^+ Exchange	Cytoskelatal functions, Smooth muscle contraction	

Signalling mechanism

The transduction of the signal through the membrane by the receptor is not completely understood. It is suggested that in the resting state, the G-protein exists as an α-β-γ- trimer with GDP occupying the site on α-subunit. Once the ligand is recognized, the receptor shifts conformation and mechanically activates the G protein, which facilitates displacement of guanosine diphosphate (GDP) by guanosine triphosphate (GTP). Binding of GTP activates the α-subunit, and the α-GTP complex then dissociates from the βγ subunits and interacts with a membrane bound effector protein (e.g. ion-channel, enzyme, transport protein, etc.). Each of the α-GTP complex can regulate one or more effectors. Depending on the type of G-protein (e.g. G_s or G_i), there is either stimulation or inhibition of the effector (See Table 6.1). The β and γ subunits are highly hydrophobic and remain associated as βγ complex with the intracellular surface of the membrane. The βγ subunits have also been shown to modulate some effectors like receptor operated K^+ channels, adenylate cyclase and phospholipase C. The activation process is terminated when hydrolysis of GTP to GDP + P_i occurs through GTPase activity of the α-subunit and the resulting reassociation of α- and βγ- subunits (Figure 6.2). Different isoforms of G proteins have different signal roles. For example, the stimulatory G_s when binds GTP, activates adenylate cyclase, and the inhibitory G_i when binds GTP, inhibits adenylate cyclase.

It has been estimated that the initial ligand-receptor interaction lasts only for a few seconds. But once activated, the G-protein action lasts for about 10 seconds, which enormously amplifies the original signal. Thus in the G-protein coupled system, occupancy of only a fraction of receptor population is enough to produce maximal response.

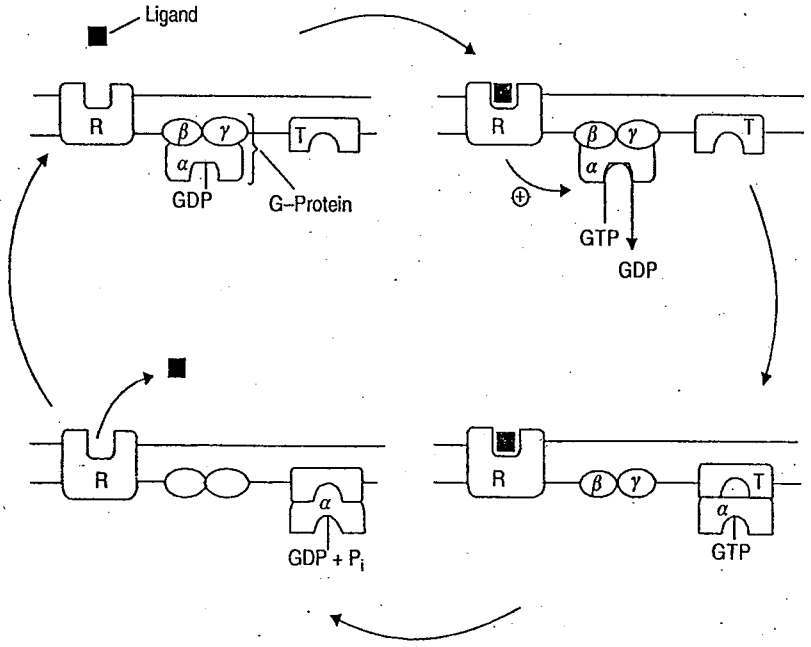


Fig. 6.2. Schematic representation of regulatory functions of G-protein (See text for detail). **Abbreviations:** R=receptor; T = target; α, β and γ = alpha, beta and gamma subunits of G-protein; GDP = guanosine diphosphate; GTP = guanosine triphosphate; P_i = inorganic phosphate group.

Effector systems

There are two different effector systems mediating signal transduction involving the G protein-coupled receptors: the enzyme-linked effector system and the ion-channel linked effector system.

1. Enzyme-linked effector systems: There are three enzyme-effector systems through which the G-protein coupled receptors function.

a. Adenylate cyclase : cAMP system: The adenylate cyclase: cAMP system is one of the most important effector systems, which has a wide variety of receptor population and produces diverse effects. In this system, the effector protein is membrane bound adenylate cyclase (adenylyl cyclase), which is either activated or inhibited by an activated α-GTP complex. There are at least ten tissue-specific adenylate cyclase isoenzymes, each with its unique pattern of regulatory responses.

Activation of adenylate cyclase enzyme by stimulatory G-protein (e.g. G_s) leads to increased synthesis of a second messenger cAMP (3', 5'-cyclic adenosine monophosphate) from ATP. Accumulation of cAMP within the cell activates cAMP-dependent protein kinases (PKA). The activated protein kinase in turn phosphorylates (transfers the terminal phosphate of ATP to a hydroxyl group on a protein) and alters the function of many enzymes, ion-channels, carrier molecules and structural proteins to manifest various pharmacological effects like glycosylation, lipolysis, inhibition of secretion/mediator release, increased contractility in heart and modulation of hormone synthesis. The intracellular effects of cAMP are terminated by degradation of cAMP to 5'-cAMP by phosphodiesterase (PDE) enzyme (Figure 6.3). Some drugs (e.g. methyl xanthine) competitively inhibit PDE and, thus, prolong the action of intracellular cAMP. Signal amplification is an important feature of adenylate cyclase: cAMP system where one ligand can lead to formation of many cAMP molecules and each catalytic subunit of protein kinase A can catalyze phosphorylation of many proteins during the life-time of the cAMP.

Receptors linked to inhibitory G-protein (G_i) suppress activity of adenylate cyclase enzyme and reduce intracellular cAMP formation. The reverse pharmacological effects occur when adenylate cyclase enzyme is inhibited. Examples of inhibitory G-protein coupled receptors include certain types of muscarinic-cholinergic receptors (e.g. M_2 receptor in heart), α_2-adrenoceptor in smooth muscle and opioid receptors.

b. Phospholipase C: IP_3-DAG system: It is another important intracellular second messenger system in which the effector protein is membrane bound phospholipase C (PLC). Activation of PLC hydrolyses the membrane phospholipid phosphatidyl inositol 4,5-bisphosphate (PIP_2) to generate

Activation of phospholipase C by various ligands is essentially similar to the activation of adenylate cyclase described above, though different G-protein subtypes are involved.

i. *Inositol triphosphate (IP_3):* In many cells, cleavage of the membrane phosphatidyl inositol 4,5-bisphosphate by phospholipase C yields the second messenger inositol-1,4,5-trisphosphate (IP_3). Once it is generated, the IP_3 due to its hydrophilic nature migrates to the cytosol and acts very effectively to mobilise Ca^{++} (third messenger in this setting) from the intracellular organellar depots such as endoplasmic reticulum apparently by binding to a receptor on the membrane. This causes an increase in free intracellular calcium concentration by 10 to 100 fold producing a range of cellular responses including modulation of ion-channels, enzymes and contractile proteins. Intracellular calcium regulates cellular activities in series of steps mainly by interaction with calcium sensor proteins such as calmodulin or troponin C and protein mediators such as protein kinase C (PK_C). Increase in the free intracellular calcium concentration is perhaps one of the most important mechanisms by which cellular effects are produced by many drugs in different types of cells. Examples of pharmacological effects of importance include smooth muscle contraction, increased force of contraction of cardiac muscle, secretion from exocrine glands and release of neurotransmitters from neurons and hormones from endocrine glands. Signal turn-off of this system includes removal of Ca^{++} from the cytosol by action of Ca^{++}-ATPase pumps and degradation of IP_3.

ii. *Diacyl glycerol (DAG):* The diacylglycerol (DAG) is another second messenger produced from the hydrolysis of phosphatidyl inositol 4,5-bisphosphate by the enzyme phospholipase C, through the same reaction that produces inositol trisphosphate (IP_3). Unlike IP_3 that is hydrophilic and diffuses from plasma membrane into the cytosol, the DAG is a hydrophobic molecule and remains within the plasma membrane. The generation of DAG in the membrane facilitates translocation of protein kinase C (PK_C) from the cytosol to the plasma membrane and its activation. The activated PK_C then controls many cellular functions by causing phosphorylation of a variety of intracellular proteins (Figure 6.3). At least six different types of PK_C have been found to be distributed unequally in different cells. Important functions ascribed to PK_C include release of hormones from many endocrine glands, increase or decrease in neurotransmitters release, contraction or relaxation of smooth muscles and inflammatory responses.

Similar to adenylate cyclase, the phospholipase C can be inhibited through inhibitory G-proteins. The responses would

(cGMP) as the second messenger from the guanosine monophosphate

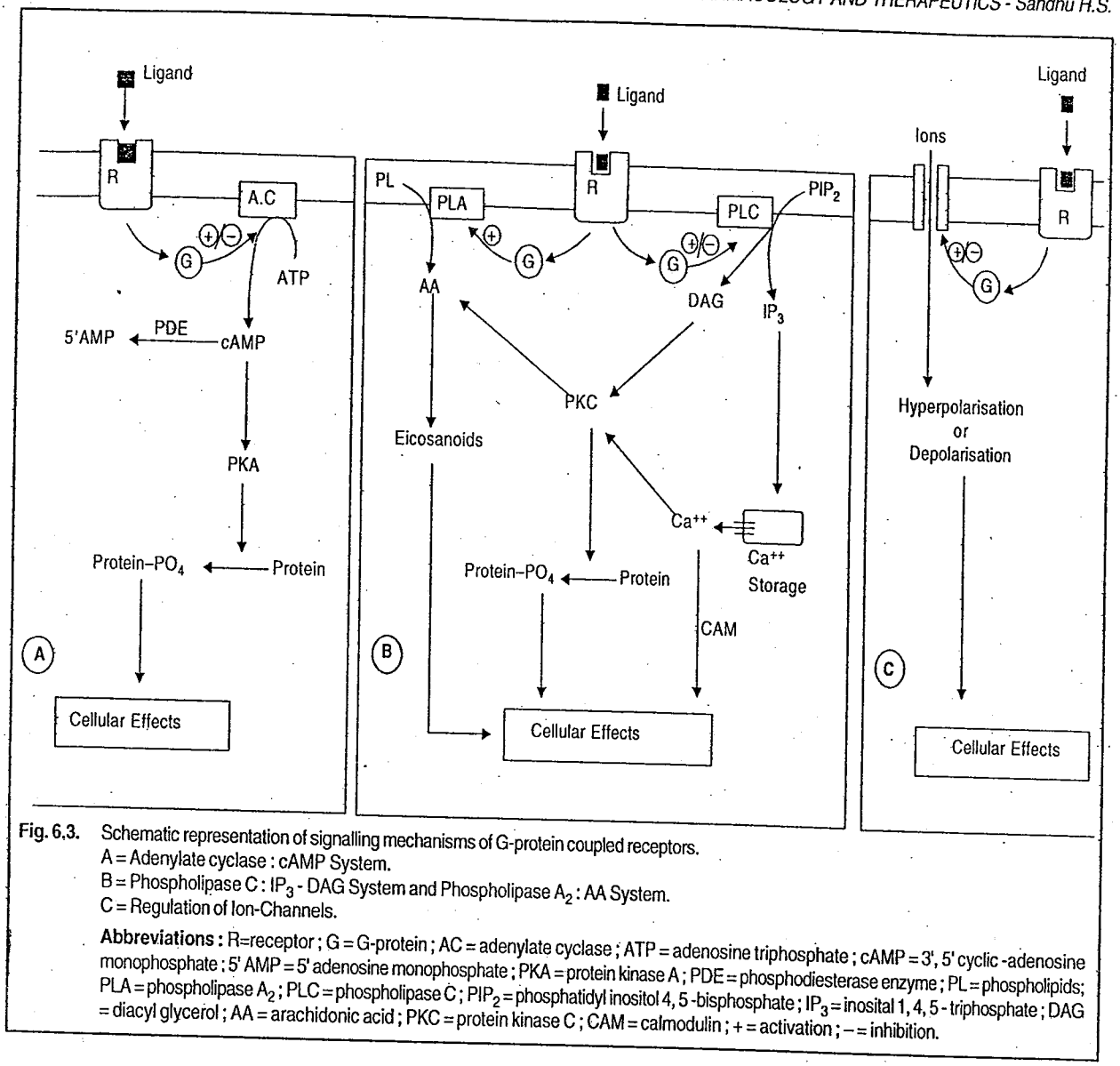


Fig. 6.3. Schematic representation of signalling mechanisms of G-protein coupled receptors.
A = Adenylate cyclase : cAMP System.
B = Phospholipase C : IP_3 - DAG System and Phospholipase A_2 : AA System.
C = Regulation of Ion-Channels.

Abbreviations : R=receptor ; G = G-protein ; AC = adenylate cyclase ; ATP = adenosine triphosphate ; cAMP = 3', 5' cyclic -adenosine monophosphate ; 5' AMP = 5' adenosine monophosphate ; PKA = protein kinase A ; PDE = phosphodiesterase enzyme ; PL = phospholipids; PLA = phospholipase A_2 ; PLC = phospholipase C ; PIP_2 = phosphatidyl inositol 4, 5 -bisphosphate ; IP_3 = inositol 1, 4, 5 - triphosphate ; DAG = diacyl glycerol ; AA = arachidonic acid ; PKC = protein kinase C ; CAM = calmodulin ; + = activation ; – = inhibition.

receptors. In this enzyme-effector system, binding of ligand to the receptor activates a G-protein that in turn stimulates phospholipase A_2 (PLA_2), a membrane bound enzyme. Activation of PLA_2 causes production of arachidonic acid (AA) from the membrane phospholipids. Arachidonic acid is further metabolised to various eicosanoids such as prostaglandins, leukotrienes and thromboxanes in a series of steps (See for detail Chapter 29). Arachidonic acid itself and its metabolites (eicosanoids) function as intracellular messengers, controlling potassium channel function in certain neurons. Histamine receptors mediate some of their actions through arachidonic acid system.

2. Ion-channel linked effector systems: G-protein coupled receptors are a category of metabotropic receptors which do not form ion channel pores rather linked to ion channels through G-protein without involving any second messenger. In this case, binding of ligand to the cell surface G-protein receptor activates a specific G-protein, which interacts directly with the ion-channel (Figure 6.3), presumably in the same way as it interacts with membrane enzymes responsible for the synthesis of second messenger. Depending on the type of G-protein (stimulatory or inhibitory), the ion-channels specific for Na^+, K^+ or Ca^{++} are either opened or closed, thereby causing hyperpolarisation, depolarisation or changes in intracellular Ca^{++} concentration leading to intracellular responses. For example, G_s opens Ca^{++} channels in myocardium and skeletal muscles, while G_i and G_o open K^+ channels in heart and smooth muscle as well as close Ca^{++} channels in neurons. Examples of receptors regulating ionic channels through G-proteins are given in Table 6.1.

III. Enzymatic Receptors

Enzymatic receptors (Enzyme-linked receptors, tyrosine kinase-linked receptors) are a group of cell surface receptors, which themselves act as enzymatic proteins. The majority of these receptors are protein kinases, often tyrosine kinases, which phosphorylate intracellular target proteins, thereby changing the physiological function of the target cells. Other receptors are serine/threonine kinases, histidine kinases and guanylyl cyclases. Phosphorylation of proteins by these receptor kinases is an important mechanism in signal transduction for regulation of several cellular activities. Enzymatic receptors are involved mainly in events controlling cell growth and differentiation and are less important for xenobiotics action.

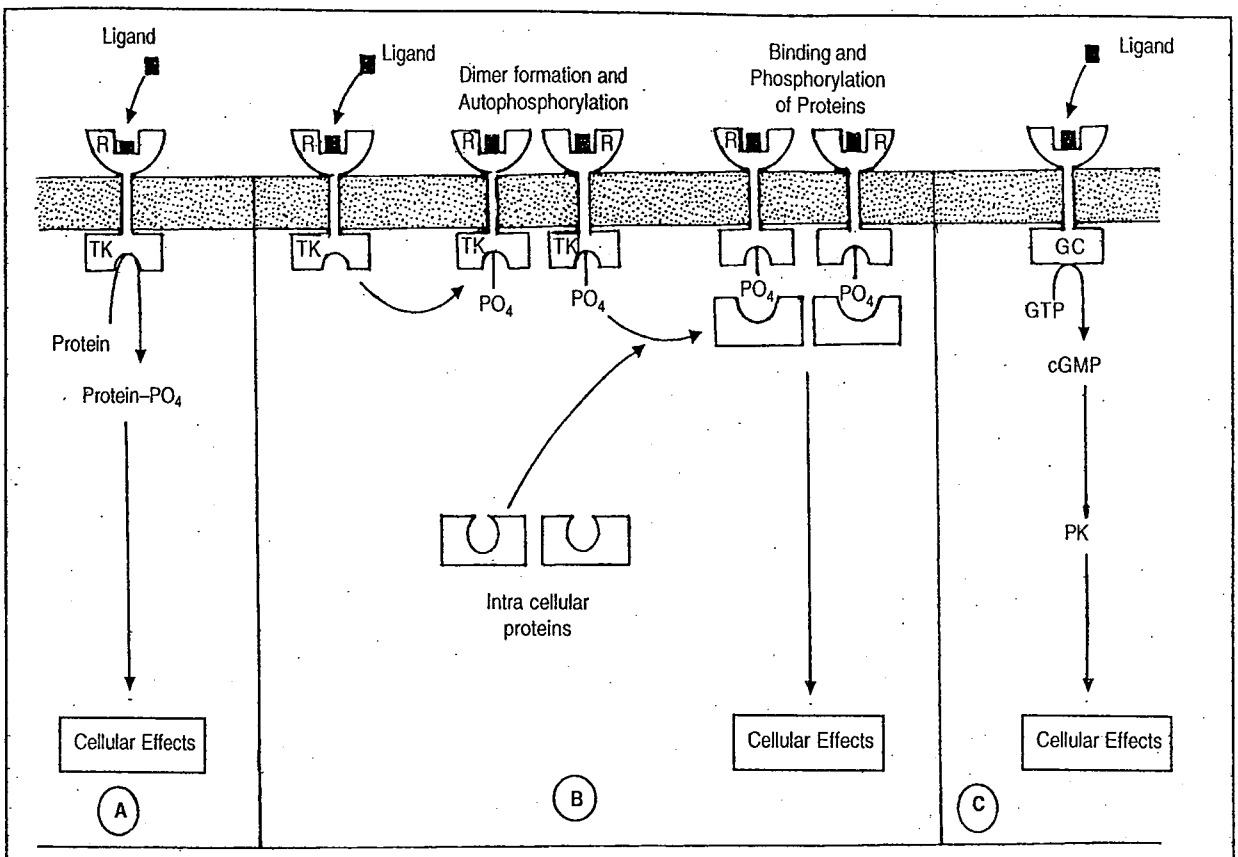


Fig. 6.4. Schematic representation of signalling mechanisms of enzymatic receptors.
A = Tyrosine-kinase linked receptor : Phosphorylation of proteins.
B = Tyrosine - kinase linked receptors : Dimerisation and autophosphorylation.
C = Guanylate cyclase - linked receptor : Generation of cGMP.
Abbreviations : R = receptor ; PO_4 = phosphate group ; TK = tyrosine kinase; GC = guanylate cyclase ; cGMP = cyclic - guanosine monophosphate; PK = protein kinase.

Molecular structure

All enzymatic receptors are membrane bound and have common pattern of structural organisation. Most receptors are single subunit receptors but some exist as multimeric complexes e.g. the insulin receptor that forms disulfide-linked dimers in the absence of hormone. The enzymatic receptors have very large extracellular and intracellular domains, with about 400 to 700 residues in each and a single hydrophobic transmembrane-spanning domain composed of 25-38 amino acids (Figure 6.6). The extracellular N-terminal region exhibits a variety of conserved elements and contains primarily a ligand-binding site to bind extracellular ligands such as a specific growth factor or hormone. The intracellular C-terminal region displays the highest level of conservation and comprises catalytic domains responsible for enzymatic activity of these receptors. In most cases, the intracellular domain is made up of protein kinases, particularly tyrosine kinase, which acts by phosphorylating target proteins. In some cases, the intracellular domain is guanylate cyclase, which synthesises the second messenger cGMP.

Signalling mechanism

a. ***Protein kinases***: When a specific ligand binds to the extracellular ligand-binding domain, the intracellular protein kinase gets activated and phosphorylates target proteins. These targets may be enzymes (including other kinases), regulatory proteins or structural proteins. Phosphorylation of target proteins may either alter their individual activities or influence their interaction with other regulatory proteins or effectors. Most tyrosine kinase receptors phosphorylates specific tyrosine residues on their specific proteins; few receptors with other protein kinases phosphorylate serine or threonine residues. Sometimes the receptor itself gets phosphorylated (autophosphorylation) on tyrosine residues. This generally follows when ligand binding leads to dimerisation of receptors (association or combination of two receptors). The autophosphorylated tyrosine residues then serve as high affinity binding sites for various other intracellular proteins. Some of these proteins are enzymes, including other protein kinases and phospholipases, which may cause IP_3 formation and Ca^{++} release and allow control of many cell functions. The mechanisms involving receptor controlled tyrosine phosphorylation play pivotal roles in diverse cellular activities including growth, differentiation, intermediary metabolism, adhesion and motility. Many tyrosine kinase receptors are involved in oncogenesis, either by gene mutation or chromosome translocation, or simply by over-expression.

b. ***Guanylate cyclase : cGMP:*** The intracellular substrate binding domain in few enzymatic receptors (e.g. atrial natriuretic peptides) is not protein kinase but is a guanylate cyclase enzyme. Ligand binding activates the guanylate cyclase that generates cyclic-guanosine monophosphate (cGMP) as the second messenger from the guanosine

triphosphate (GTP). Increased intracellular cGMP in turn activates cGMP-dependent protein kinase and modulates cellular activity (Figure 6.4). For most of the effects produced, cGMP seems to be inhibitory in nature (e.g. produces relaxation of vascular smooth muscle).

IV. Receptors Regulating DNA Transcription/ Steroid Receptors

Receptors regulating DNA transcription (Steroid receptors, intracellular receptors) are located inside the cell rather than on its cell membrane. These receptors directly interact with and control the expression of genomic DNA in the nucleus. Ligands for these intracellular receptors include cell-permeant or lipophilic substances such as steroidal hormones, thyroid hormones, and vitamins A and D. Cellular effects are produced mainly as a result of protein synthesis; therefore, the action of these receptors is slow in onset.

Molecular structure

The receptors regulating DNA transcription are primarily intracellular soluble proteins located either in the cytoplasm or in the nucleus (mainly). The receptors for steroidal hormones are primarily large monomeric proteins of 400 to 1000 residues consisting of three largely independent domains. The region nearest to the carboxyl-terminus binds hormone and serves as ligand binding domain (Figure 6.6). It is moderately conserved in sequence and highly conserved in structure between the various nuclear receptors. The central highly conserved region of about 60 residues constitutes the DNA binding domain of the receptor. It contains two loops of about 15 residues each, called the zinc fingers (so called because they consist of a cluster of four cysteine residues surrounding the zinc atom), which are believed to wrap around the specific sequence of DNA helix. The function of third domain consisting of the amino-terminus is less well defined, but is believed that it performs some regulatory functions and is called regulatory domain or transcriptional control domain. This region is highly variable in sequence between various nuclear receptors. Both the DNA-binding domain and the ligand binding domain contribute to the dimerization interface of the receptor and in addition bind co-activator and co-repressor proteins.

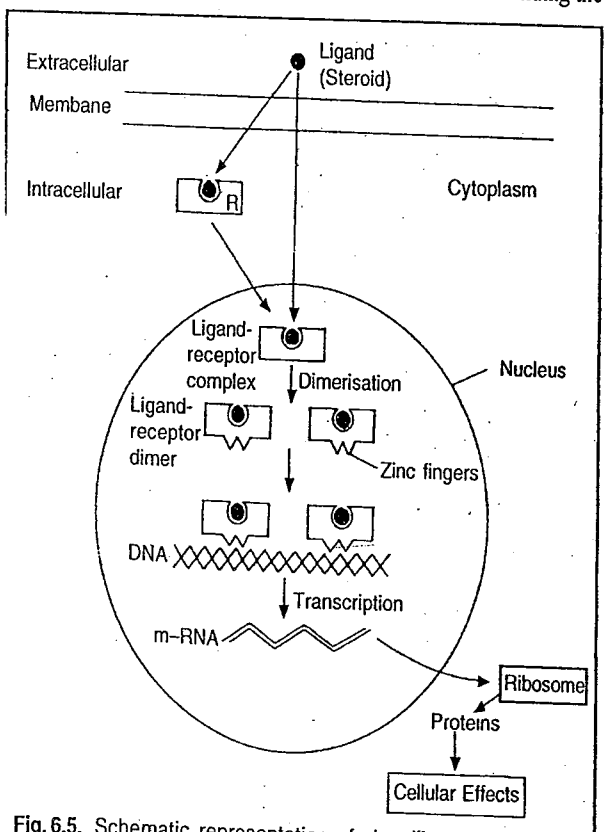


Fig. 6.5. Schematic representation of signalling mechanism of receptors regulating DNA transcription.
Abbreviation : R = receptor ; DNA = deoxyribonucleic acid; mRNA = messenger ribonucleic acid.

Signalling mechanism

When a lipophilic ligand such as a steroid hormone diffuses past the cell membrane and binds to the intracellular receptor located in the cytosol or nucleus of the cell, a conformational change occurs in the receptor and a ligand-receptor complex is formed. If the binding between ligand and receptor occurs in cytosol, then the ligand-receptor complex first migrates to the nucleus and then carries out the future events. In the nucleus, the ligand-receptor complex triggers a number of downstream events to alter the gene expression. It is believed to unfold and expose the normally buried DNA binding domain and remove the inhibitory influence on it. Binding of the ligand to its receptor also promotes dimerisation process i.e. linking of two receptor complexes. The ligand-receptor dimer formation is considered an essential component for steroid hormones as it

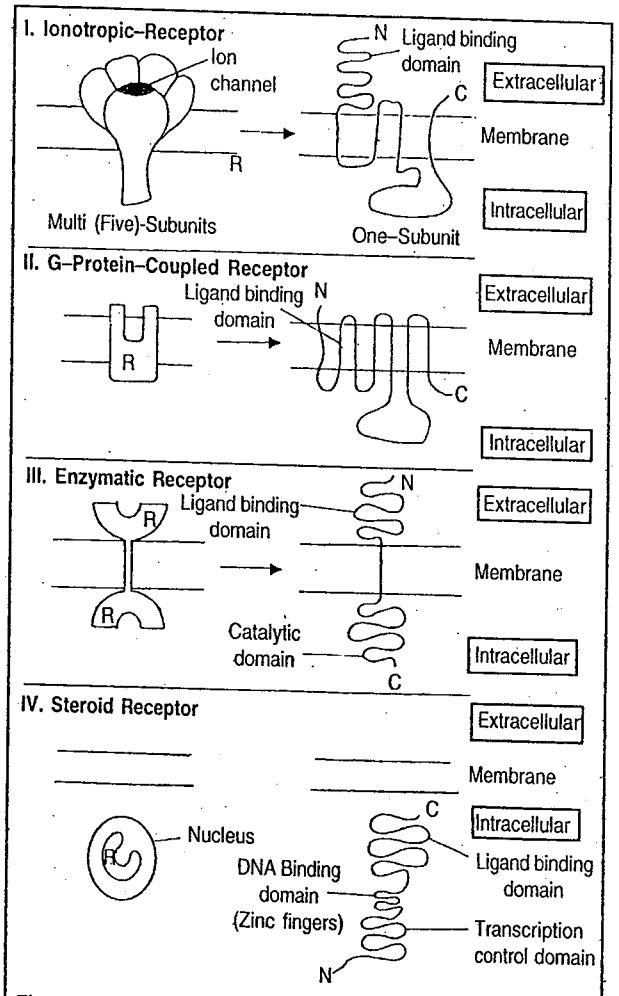


Fig. 6.6. Schematic representation of molecular structures of four receptor superfamilies (see text for details).
Abbreviation : R = receptor ; N = amino/nitrogen terminus ; C = carboxyl/carbon terminus.

Table 6.2: Comparative features of 4 superfamilies of drug receptors.

Feature	Ionotropic-receptor	G-protein coupled receptor	Enzymatic receptor	Receptor regulating DNA transcription
Location	Membrane	Membrane	Membrane	Intracellular
Number of membrane spanning domains	Four	Seven	One	Nil
Effector	Ion-channel	Enzyme or Ion-channel	Enzyme	Gene transcription
Coupling	Direct	G-protein	Direct	Via DNA
Response time	Milliseconds	Seconds	Minutes	Hours to days
Examples	Nicotinic-R $GABA_A$-R NMDA-R AMPA-R 5-HT_3-R P_2X ATP-R	Muscarinic-R Adrenergic- R Histamine-R Opioid -R Adenosine -R Angiotensin-R Glucagon-R 5-HT-R (except 5-HT_3-R) Chemokine-R	Insulin- R Growth factors-R Erythropoietin-R Cytokine -R Atrial-natriuretic peptide-R	Glucocorticoid-R Mineralocorticoid-R Oestrogen-R Androgen-R Thyroid hormone-R Vitamin A-R Vitamin D-R

Abbreviations: GABA = γ-aminobutyric acid; 5-HT = 5-hydroxytryptamine; R = receptor; NMDA = N-methyl-D-aspartate; AMPA = α-amino-3-hydroxy-5-methyl isoxazole propionic acid.

recognises and binds to certain well-defined regions in the nuclear DNA. The activation or inhibition of DNA then results in up or down regulation of gene expression altering production of specific m-RNA. The newly synthesised m-RNA moves out of the nucleus to the ribosome and modulates synthesis of specific proteins, which regulate activity of target cells (Figure 6.5). Different ligands are able to activate different genes and, thus, initiate completely different pattern of protein synthesis and produce different effects. These receptors also work in concert with several other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis and metabolism of the organism.

The effect of steroid receptors is the slowest in its time course of action with a lag period of 30 minutes to several hours or days, the time required for synthesis of proteins. Additionally, the effects of these receptors can persist for hours or days after the ligand has been removed because of slower turnover of newly synthesised proteins or enzymes, which can remain active in body for hours or days after they have been synthesised. All steroidal hormones mediate action through these receptors.

Receptor Subtypes

As the diversity and selectivity of drugs have increased, it has become clear that multiple subtypes of receptors exist within many previously defined classes of receptors. Subtypes of same class of receptors (e.g. α_1 and α_2, and β_1, β_2 and β_3 adrenoceptors) have been identified, which display fundamental differences in their biochemical regulatory activities. Such differences allow an agonist to produce unique responses in specific cells or tissues. Different receptor subtypes may also be found within a single tissue at different stages of growth and development, reflecting altered needs of the organism. Identification of receptor subtypes has greatly facilitated the development of a number of therapeutic agents which have selectivity for specific types or subtypes of receptors and limit the frequency or intensity of unwanted effects.

DRUG-RECEPTOR INTERACTIONS

Many drugs exert their pharmacological effects by interacting with specific receptors in the body. This drug-receptor interaction leads to a conformational change in the macromolecule, which in turn triggers a sequence of events resulting in a response of the tissue or organ. In relation to drug-receptor interaction, following terms are commonly used:

a. **Drug action:** Drug action is the biochemical or physiological mechanism(s) by which a drug produces a biological response in living organisms. It starts with the initial combination of drug with its receptor resulting in a conformational change in the latter (in case of agonist) or prevention of a conformational change through exclusion of the agonist (in case of antagonist) resulting in a series of biochemical and metabolic changes in the cell.

b. **Drug effect:** Drug effect is the ultimate observable change in the biological function of a cell or tissue brought about as a consequence of drug action. For example, the action of digitalis is to produce inhibition of sodium pump (Na^+-K^+-ATPase) in the myocardial cells and the effect is the myocardial contraction. Similarly, the action of NSAIDs is to inhibit cyclooxygenase enzyme and the effect is the analgesia. Many times the terms drug action and drug effect are used interchangeably, but they are not synonyms and should not be used for the same purpose.

c. **Affinity:** Affinity is the ability of a drug to combine with its receptor. In general, high affinity drug binding results from greater intermolecular force between the drug and its receptor while low affinity drug binding involves lesser intermolecular force between the drug and its receptor. High affinity drug binding also implies that a relatively low concentration of a drug is adequate to maximally occupy a binding site and trigger a pharmacological response in contrast to low affinity binding that requires a relatively

high concentration of a drug to affect binding and produce the pharmacological response. The affinity of a drug for its receptor depends on its chemical structure.

d. **Intrinsic activity:** Intrinsic activity (IA) or efficacy refers to the relative ability a drug to evoke a pharmacological response (drug effect) on combining with the receptor. High efficacy drugs can produce the maximal response while occupying a relatively low proportion of the receptors in that system, whereas low efficacy drugs may not be able to produce the same maximal response, even when they occupy the entire receptor population. Similar to affinity, the intrinsic activity of a drug is its inherent property and depends on the chemical structure of drug.

TYPES OF DRUG

Receptors can be activated or inactivated by drugs, resulting in stimulation or inhibition of a biological response, respectively. Depending on its interaction with a receptor and the consequent pharmacological effect, the drugs have been categorized into different types.

1. Agonist

Agonist is a drug that interacts with a specific receptor(s) and elicits an observable positive response. The agonist can activate the receptor because it resembles the endogenous ligands such as a neurotransmitter or hormone. An agonist has both affinity and intrinsic activity (Table 6.3). Agonists may be further classified as full agonists and partial agonists.

i. ***Full agonist:*** It is an agonist that binds to and activates a receptor to elicit the greatest response of which the respective tissue is capable of. For a full agonist, the intrinsic activity (IA) is equal to unity or 1. For practical purposes, the terms full agonist and agonist are used interchangeably. Examples of full agonist include isoprenaline that mimics the action of endogenous ligand epinephrine at β-adrenoceptors and morphine that mimics the actions of endorphins at μ-opioid receptors.

ii. ***Partial agonist:*** It an agonist that acts on the same receptor as a full agonist but regardless of its dose it cannot elicit the greatest response of which the respective tissue is capable of. A partial agonist has high affinity for the receptor, but possesses a low to moderate intrinsic activity. For a partial agonist, the intrinsic activity is always less than 1 (IA between 0 to 1). A partial agonist when used alone acts as an agonist to produce the response (lower than full agonist), but in presence of a full agonist/agonist, it acts as an antagonist (lowers effect of full agonist as it competes with full agonist for receptor occupancy and decreases availability of free receptors to it). A drug that acts as a partial agonist in one tissue may act as a full agonist in another. Buprenorphine, a semi-synthetic opiate, has partial agonist actions at the mu opioid receptor.

2. Antagonist

Antagonist is a drug that interacts with a specific receptor(s) or other part of the effector mechanism to inhibit action of an agonist. Such an agent is devoid of intrinsic activity and does not provoke biological response itself upon binding to the receptor, but blocks or dampens agonist-mediated responses. The antagonists, thus possess affinity for the receptors, but have no intrinsic activity (IA = 0). Antagonists mediate their effects by binding to the active site or to allosteric sites on receptors, or they may interact at unique binding sites not normally involved in the biological regulation of the receptor's activity. Depending on the type of interaction with the receptors, antagonists may be further classified as competitive antagonists and non-competitive antagonists (see for detail, antagonism). Receptor antagonists are often called blockers. Examples of receptor antagonists include naloxone, an opioid receptor antagonist, which blocks effect of morphine and propranolol, a β-adrenoceptors antagonist, which blocks action of isoprenaline. Similarly, atropine antagonises effect of acetylcholine on muscarinic receptors and flumazenil blocks action of diazepam on benzodiazepine receptors.

3. Inverse agonist

Inverse agonist is a drug that interacts with the same receptor as the agonist, but it produces a response specifically opposite to that of the agonist. Inverse agonist has affinity but it possesses intrinsic activity with a minus sign (IA = 0 to –1). For example, benzodiazepines like diazepam cause sedation, anxiolysis and muscle relaxation through their agonist effects at the $GABA_A$ receptor, while β-carbolines (inverse agonist) like dimethoxyethyl carbomethoxy-β-carboline (DMCM) at this receptor produces stimulation, anxiety, increased muscle tone, etc. The β-carbolines, in addition to their direct actions, can block effects of benzodiazepines. Some drugs previously classified as antagonists are now beginning to be reclassified as inverse agonists because they not only block the effects of binding agonists like a classical antagonist but also cause a distinct set of downstream biological responses e.g. histamine H_1- receptor antagonists.

4. Agonist-antagonist

Agonist-antagonist is an agent that acts simultaneously on a mixed group of receptors with an agonist action on one set and an antagonist action on another set. For example, nalorphine, an opioid, activates κ-opioid receptor but blocks μ-opioid receptor. Similarly buprenorphine, another opioid, has partial agonist actions at the mu opioid receptor and antagonist actions at the kappa opioid receptor.

Table 6.3: Comparative feature of various types of drugs in relation to drug-receptor interaction.

Activity	Types of drug			
	Agonist	Partial agonist	Antagonist	Inverse agonist
Affinity	+++	+++	+++	+++
Efficacy	+++	+	0	-
With agonist	Addition or Synergism	Antagonism	Antagonism	Antagonism

+ = Positive effect; 0 = no effect; - = negative effect

5. Other types

Some other less frequently used terms for a drug in pharmacology include superagonist, co-agonist, irreversible agonist and selective agonist. The term superagonist is used for agonist that produces a maximal response greater than the endogenous agonist for the target receptor, and thus has an efficacy of more

than 100%. A co-agonist works with other agonists to produce the desired effect together e.g. NMDA receptor activation requires the binding of both of its glutamate and glycine co-agonists. An irreversible agonist binds permanently to a receptor in such a manner that the receptor is permanently activated; this causes a brief burst of agonist activity, followed by desensitization and internalization of the receptor with long-term effect more like that of an antagonist. A selective agonist binds and activates only one certain type of receptor.

DRUG-RECEPTOR INTERACTION AND DRUG EFFECT

The concept of a receptor as a selective locus of action was pioneered more than a century ago, but even today the receptor module remains an important concept in pharmacology and many other branches of biology. A large number of xenobiotics are known to act through specific receptors and several antagonists have been designed to neutralise their action. The magnitudes of response elicited by a drug-receptor interaction vary widely between the drugs having same or similar actions and still a lot remains to be explained. Several theories have been put forward to explain the nature of drug-receptor interactions and their relationships to the pharmacological response produced, but none of these theories satisfactorily explain the behaviour of all drugs. Some important receptor theories which seem to reasonably explain the action of different types of drug are discussed below.

1. Drug-receptor theory

The concept of receptors as the targets of drug action is credited to the independent work of Paul Ehrlich (1845-1915) and John Newport Langley (1952-1926). In 1905, Langley introduced the concept of a receptive substance on the surface of skeletal muscle that mediated the action of a drug and around the same time Ehrlich worked on the selectivity of agents. Their work led to a drug-receptor theory that explained specific action of a drug with a single receptor using a Lock and Key analogy, the concept first postulated in 1894 by Emil Fischer for enzyme and substrate interaction. In this analogy, the structure of the drug (key) is related to the correlative structure of the receptor (lock), and just one correctly sized key can fit in one lock so also one drug is specific for one receptor type (Figure 6.7). Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized drug molecules) do not fit into the keyhole (active site) of the lock (receptor).

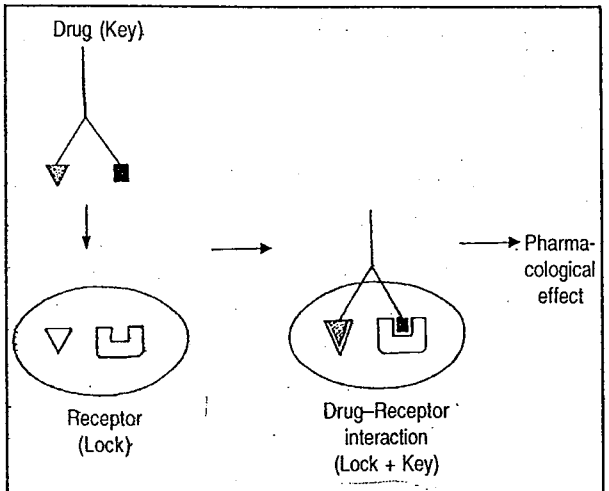


Fig. 6.7. Drug-receptor theory suggesting a mutual molding of drug and receptor like a lock and its matching key.

Postulates of receptor theory: Following assumptions have been made to explain the drug action on the basis of drug-receptor theory.

a. The drug must reach the receptor in order to produce a pharmacological response.
b. The drug is specific to its matching receptor.
c. The receptor must possess structural and steric specificity.
d. The receptors are saturable and finite (limited number of binding sites).
e. The receptor must possess high affinity for its ligands.
f. The drug-receptor specificity depends on the chemical structure of the drug.

The drug-receptor theory provides only the basic understanding of the drug-receptor interaction and does not explain how and in what magnitude the action is produced.

2. Receptor occupancy theory

Receptor occupancy theory, which describes the concept of agonists and competitive antagonists, was built on the work of J. N. Langley and put forward in 1937 by a British pharmacologist Alfred Joseph Clark (1885-1941). It is based on the law of mass-action kinetics and attempts to link the action of a drug to the proportion of receptors occupied by that drug at equilibrium. The theory postulates that the magnitude of response produced by a drug is directly proportional to the number of receptors occupied, and the maximum response is elicited once all receptors are occupied at equilibrium. The theory was based on the presumption that drug exerts an 'all or none' action on each receptor (i.e. either a receptor is fully activated or not at all; there is no partial activation) and that each occupied receptor delivers a constant unit response, which when summated in mathematical fashion gives a linear proportional response. He demonstrated that for many drugs the relationship between drug concentration and biological effect corresponded to a hyperbolic curve, similar to that representing the adsorption of a gas onto a metal surface. Clark, together with J. H. Gaddum (1900-1965), was the first to introduce the log concentration–effect curve and described the now-familiar 'parallel shift' of the log concentration–effect curve produced by a competitive antagonist.

The receptor occupancy theory gave a fundamental concept, but it is an oversimplified view. The theory does not explain the action of partial agonists which are unable to elicit maximal response of which the tissue is capable of even after full receptor occupancy. Similarly, this theory cannot explain action of agonists like epinephrine and histamine, which can produce maximal response even by occupying a small fraction (<1%) of receptors.

To overcome above-mentioned problems, E.J. Ariens in 1954 and R. Stephenson in 1956 modified the receptor occupancy theory and introduced the concept of efficacy. The modified theory postulated that different drugs have different efficacies or intrinsic activities to induce a pharmacological response. A drug of high efficacy elicits a maximal response after occupying only a small proportion of the receptor population, whereas a drug of lower efficacy has to occupy a greater proportion of receptors to elicit an equal response. The partial agonist fails to induce maximal response even when all receptors are occupied because its efficacy is low to allow the maximal response. It was proposed in the modified theory

that efficacy is small for partial agonists, indefinitely large for full agonists and zero for competitive antagonists.

3. Rate theory

Rate theory of drug action was introduced by W. D. M. Paton in 1961. The theory postulates that occupation of a receptor alone is of no importance to the action of drug, instead it is the rate of drug-receptor complex formation and rate of complex breakdown that determine the intrinsic activity of drug. The act of making a drug-receptor complex provides a unit of stimulus to the cell and the greater the number of drug-receptor complex formation per unit time, the greater is the stimulus to the cell. However for the sustenance of a response, the complex formed has to be broken and remade. The more rapidly the complex dissociates, the more rapidly can new association takes place. According to this hypothesis, the agonists have high rate of association and dissociation and, thus, have high turnover rate. In contrast, antagonists make complex rapidly but dissociate very slowly (i.e. they produce a stable drug-receptor complex) and partial agonists form complex rapidly but dissociate moderately. As the rate of association (affinity) for agonist, partial agonist and antagonist is high, it is the rate of dissociation that mainly determines the efficacy or intrinsic activity of a drug. In other words, it is the rate of dissociation that primarily determines the magnitude of response because it varies between different types of drug, in contrast to the rate of association or affinity that remains same for all types of drug.

According to the receptor occupancy theory, the interaction between the drug (D) and the receptor (R) is governed by law of mass action, and the resultant effect (E) depends on the stimulus provided by the drug-receptor complex (DR):

$$D + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DR \xrightarrow{k_3} E$$

The properties of a drug can then be specified by two rate constants: k_1, the association rate constant and k_2, the dissociation rate constant. The ratio k_1/k_2 (the rate at which complex is formed compared to the rate at which complex is dissociated) corresponds to affinity and the ratio k_2/k_1 (the rate at which complex is dissociated compared to the rate at which complex is formed) corresponds to reciprocal of 'affinity', the dissociation. The intrinsic activity (efficacy) can be measured by k_3 i.e. the rate at which an agonist evokes a pharmacological response on combining with a receptor. According to the theory, the value of k_1/k_2 (affinity) remains high for all types of drug, and the value of k_2/k_1 (dissociation) mainly determines the efficacy and whether the drug is an agonist (k_2 high), a partial agonist (k_2 moderate) or an antagonist (k_2 low or zero).

4. Drug induced protein changes theory

Drug induced protein changes theory suggests that drugs elicit their pharmacological effects by inducing certain temporary structural changes in the protein constituents at receptor site. When an agonist combines with an active site on a receptor, the receptor becomes activated, so triggering a response from the cell. When the drug leaves, the receptor returns to its non-activated original form. Antagonists also produce structural changes at receptor site by binding to either the active site or the allosteric site adjacent to active site. The antagonists binding either obscure or distort the active site as a result of which agonists can no longer complex with the receptor.

5. Two state receptor theory

The two-state receptor theory was put forward by J.W. Black and P. Leff in 1983 to describe the interaction between ligand and receptor. The theory suggests that a receptor exists in two interchangeable conformational forms, the active (R_a) and the inactive (R_i) forms. There is a dynamic equilibrium between the active and inactive forms of the receptor and both forms can bind to the drug. The active form is capable of generating pharmacological response, while inactive state does not produce response. Under normal conditions, when no ligand is present (resting state), the equilibrium favours predominantly towards the inactive state. The extent to which the equilibrium is perturbed is determined by the relative affinity of drug for the two conformations. According to the hypothesis, various types of drug produce their effect in following ways:

a. The agonist has higher affinity for the active form of receptor than the inactive form. Therefore, it drives the equilibrium to the active state (Figure 6.8) and produces pharmacological effect. The larger the drug affinity for the active form, the greater is the drug's efficacy. Thus, a full agonist has the highest affinity for the active state, so that at saturation concentration virtually 100% of receptors are in the active state.

b. The partial agonist has only slightly greater affinity for the active state than the inactive state. It, therefore, marginally shifts equilibrium towards the active state. Thus, even at saturation concentration, only a proportion of receptors exist in active state that produces response much lower than that of the full agonist.

c. The antagonist has equal affinity for both inactive and active forms. Therefore, it binds to receptors and keeps the conformational equilibrium undisturbed i.e. predominantly in inactive state. It does not allow shifting of equilibrium from the resting state even when binding approaches saturation. It simply occupies receptors and prevents binding of an agonist i.e. serves as competitive antagonist.

d. The two-state receptor theory also explains the phenomenon of inverse agonism, which occurs with benzodiazepines receptors. For this, it is postulated that an inverse agonist has a preference for the inactive receptor and it shifts the receptor equilibrium from resting state (predominantly inactive) to more inactive state. Therefore, the agonist with preferential affinity for active form will produce positive effect (e.g. benzodiazepines) and the inverse agonist with preferential affinity for inactive form will produce a negative effect (e.g. β-carbolines). Therefore, depending on the type of drug, the equilibrium can be shifted in either direction, producing opposite effects (Figure 6.8).

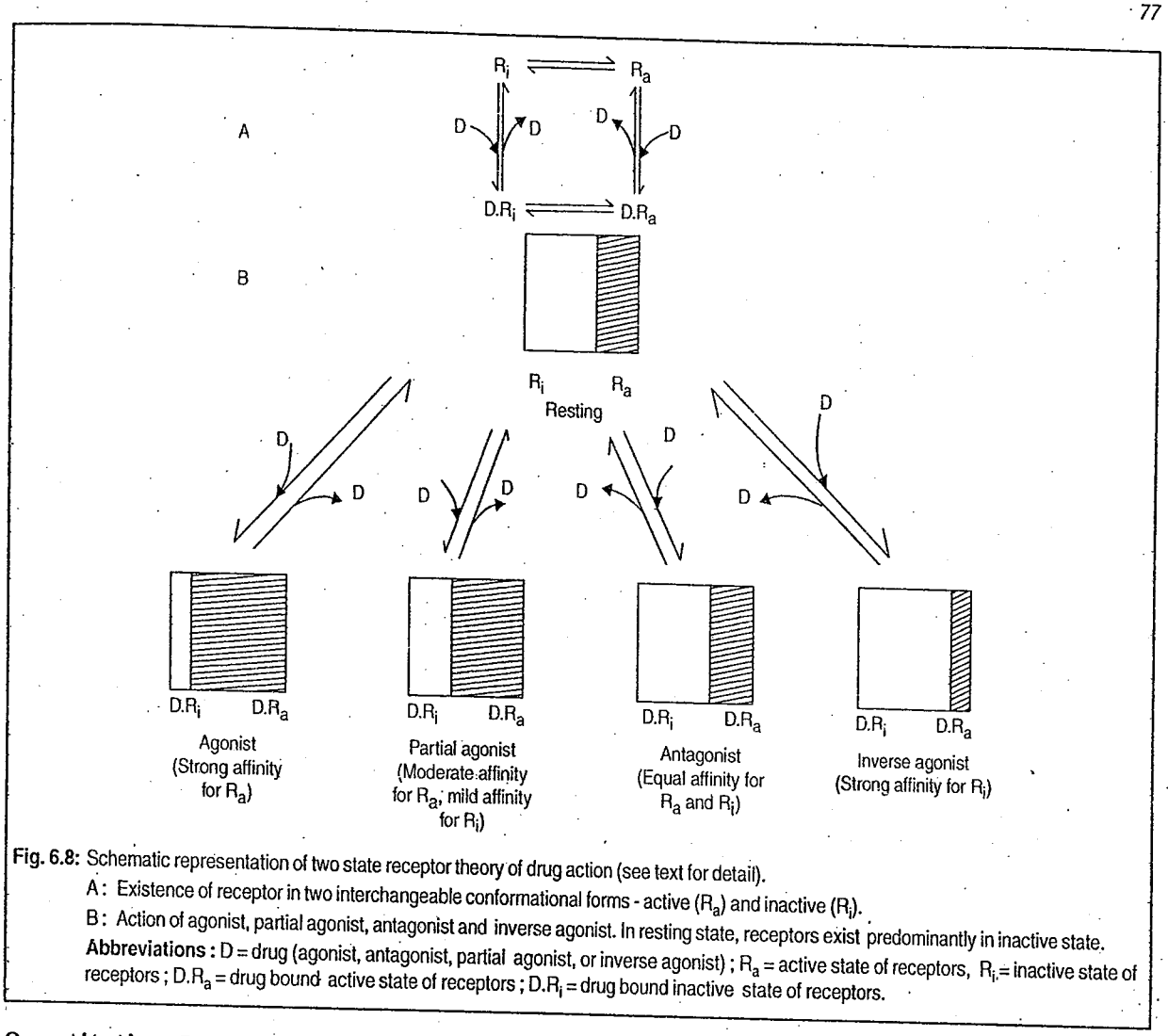


Fig. 6.8: Schematic representation of two state receptor theory of drug action (see text for detail).
A: Existence of receptor in two interchangeable conformational forms - active (R_a) and inactive (R_i).
B: Action of agonist, partial agonist, antagonist and inverse agonist. In resting state, receptors exist predominantly in inactive state.
Abbreviations: D = drug (agonist, antagonist, partial agonist, or inverse agonist); R_a = active state of receptors, R_i = inactive state of receptors; $D.R_a$ = drug bound active state of receptors; $D.R_i$ = drug bound inactive state of receptors.

Quantitative Description of Drug-Receptor Interaction and Drug Action

If one assumes that an agonist drug interacts reversibly with its receptor, than the resultant effect is proportional to the number of receptors occupied and that a maximum effect occurs when all receptors are occupied, as in the original models of A.J. Clark and W.D.M. Paton. The reaction scheme can be written as:

$$\text{Drug (D)} + \text{Receptor (R)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Drug-receptor complex (DR)} \xrightarrow{k_3} \text{Effect (E)}$$

The above reaction sequence is analogous to the interaction of a substrate with its enzyme. The magnitude of effect can be analysed in the manner similar to that for enzyme product formation according to the Michaelis-Menton equation

$$E = \frac{E_{max}\,(D)}{K_D + (D)}$$

Where, E is the effect, E_{max} is the maximal effect, (D) is the free drug concentration, and K_D is the dissociation constant for drug-receptor complex (equal to k_2/k_1). The fraction of receptors that are occupied by drug is equal to $(D)/[(K_D + (D)]$. In this equation, there are two constants for a given drug i.e. K_D and E_{max}. On plotting the two variables of the equation i.e. E and (D), the dose effect curve will be hyperbolic [(D) vs. E] or sigmoid [log (D) vs. E]. When there is linear relationship between occupancy and response, as in the original model of A. J. Clark, the concentration of an agonist at which it is half maximally effective (EC_{50}) is equal to its K_D (K_D can also be defined as the drug concentration where effect is ½ E_{max}). Reciprocal of dissociation constant (K_D) gives the affinity ($1/K_D$), which is a measure of the affinity of the drug for its receptor i.e. more the value of K_D, less would be the affinity, and vice versa.

Data analysis can be further facilitated if a linear form of equation (instead of hyperbolic or sigmoid) is taken. This is obtained by taking reciprocal of both sides called double reciprocal plot or Line-Weaver Burk plot (Figure 6.9).

$$E = \frac{E_{max}\,(D)}{K_D + (D)}$$

Taking reciprocal of both sides

$$\frac{1}{E} = \frac{K_D}{E_{max}\,(D)} + \frac{(D)}{E_{max}\,(D)}$$

$$\frac{1}{E} = \frac{K_D}{E_{max}\,(D)} + \frac{1}{E_{max}}$$

A plot of 1/E versus 1/D yields a straight line that intersects the Y-axis at $1/E_{max}$ and this has a slope equal to K_D/E_{max} (Figure 6.9). Extrapolation of this line to the X- axis yields the value of intercept shown to by $-1/K_D$. Thus, value of K_D and the maximal effect can be readily calculated from such a plot. This representation is particularly useful for the analysis of drug antagonism.

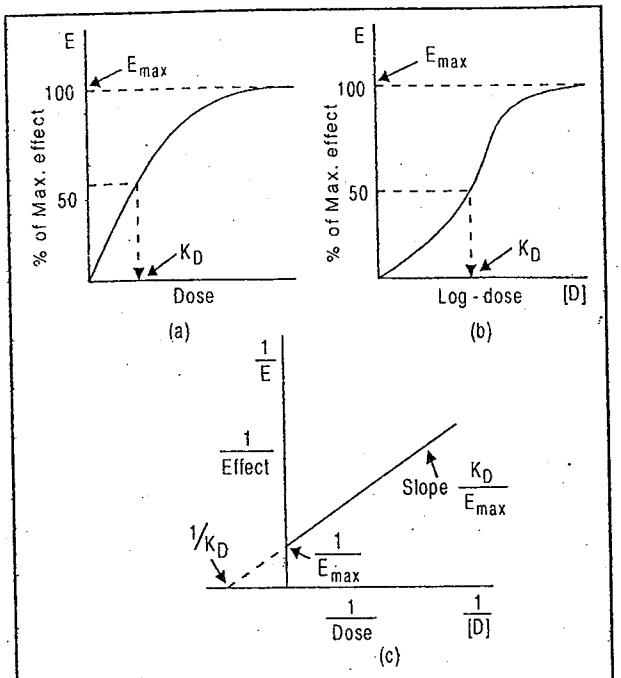


Fig. 6.9. Dose-response curve showing dissociation constant. A : Hyperbolic curve, B : sigmoid curve, C : Double reciprocal plot depicting linear form of curve.

REGULATION OF RECEPTORS

Receptors exist in a dynamic state. Their density and efficacy vary from animal to animal, and may change quantitatively even in the same animal during the course of drug therapy. The clinician must be aware of such possibilities and should change the dose or the choice of drug during the treatment, depending on the varieties of response observed in the patient.

Change in Number of Receptors

The receptor number does not remain constant but changes according to the physiological and pathological situations. Change in the receptor population can be considered as a homeostatic mechanism of the tissue with the aim to maintain the on going tissue function. Alterations in the number of receptors modify the physiological response produced by endogenous ligands (e.g. hormones and neurotransmitters) as well as the pharmacological response to drugs.

Down-regulation: Downregulation is the process by which a cell decreases the quantity of a cellular component such as a receptor or protein in response to an external variable. Down-regulation of receptors is an important phenomenon in pharmacology that generally causes diminished drug effects. The process of down regulation of receptors occurs when the tissues are continuously exposed to an agonist causing their overactivation. In such a situation, when a drug binds to its receptor on the surface of a cell, the drug-receptor complex undergoes endocytosis (internalisation) and is subsequently metabolised by intracellular lysosomal enzymes. The internalization of the drug molecules provides a pathway for degradation of the drug as well as for regulation of the number of sites which are available for binding on the cell surface. When this process occurs at a rate faster than synthesis of the receptors, the total number of cell surface receptors is reduced. Over time, this self-induced loss of target cell receptors for drugs reduces the target cell's sensitivity to the elevated drug concentration. Down-regulation phenomenon mostly leads to desensitization or refractoriness (diminished responsiveness of tissue) and is an example of a locally acting negative feedback mechanism. Receptor down-regulation is particularly exhibited by the tyrosine protein kinase receptors. This is commonly seen with continuous exposure to the hormone insulin. Alpha- and beta- adrenoceptors also show down-regulation phenomenon following the continued use of catecholamines.

Up-regulation: Up-regulation of receptors is the process by which body increases the number of receptors in a tissue. It is usually seen when receptors in a tissue are continuously deprived of their ligand for a prolonged period, either by the process of denervation of the tissues (for endogenous ligands) or continued use of an antagonist. Due to the prolonged inactivation of receptors, the tissues cannot perform properly and to overcome the deficiency body starts synthesising new receptors at a rapid pace. Up-regulation of receptors generally causes enhanced responsiveness of tissues responsible for a state of supersensitization (enhanced responsiveness of tissues). The phenomenon of up-regulation of receptors may be responsible for a rebound hypertension in humans following withdrawal of beta-adrenoceptor blockers.

The dynamic natures of receptors in a tissue are not necessarily just alterations in the numbers of receptors, but types and activities of receptors may also change. Over- or under-stimulation of one type of receptor can also have profound effects on the other type of receptors called the heterologous desensitization or supersensitization.

Change in Sensitivity of Receptors

Desensitization: Desensitization is the reduction or abolition of tissue response to a drug. Desensitization may occur due to down-regulation of receptors or depletion of essential intermediate substances (e.g. depletion of norepinephrine from adrenergic nerve terminals by continued use of amfetamine). Desensitization may also be seen after masking or covalent modification (e.g. phosphorylation) of receptors. In some cases, desensitization is limited only to agonists of one receptor after they are being repeatedly administered called the homologous desensitization. In other situations, response to all agents which act through different receptors, but produce same overt response is decreased by exposure to any one of these agents called the heterologous desensitization. Heterologous desensitization is often seen when different receptors producing same type of effect use a single signalling pathway. For example, both histamine and acetylcholine contract intestinal smooth muscle via the phospholipase C: IP_3-DAG signalling pathway. Continued use of one agent resulting in desensitization may also lead to decreased response to other drug.

Terms related to desensitization

Tachyphylaxis: The term tachyphylaxis is conventionally used to describe a rapid appearance of progressive decrease in tissue response (desensitization) following repetitive administration of a drug over a short period of time. Increasing the dose of the drug will not increase the pharmacological response. Repeated doses of ephedrine display tachyphylaxis, since it is an indirectly acting sympathomimetic amine and its repeated doses cause depletion of norepinephrine from the nerve terminal. Dobutamine, a direct-acting $beta_1$ adrenoceptor agonist used in congestive heart failure, also demonstrate tachyphylaxis. Many times, the terms tachyphylaxis

and desensitization are used synonymously, but actually they carry different meanings. The term tachyphylaxis is used mainly for reduction in response on successive administration of same dose of an agonist (see also ephedrine, Chapter 11), whereas desensitization is a broader term indicating reduction in response following administration of a single (large) dose or repetitive doses of same or similar agonists (homologous desensitization) or different agonists (heterologous desensitization).

Tolerance: The term tolerance is conventionally used to describe a gradual decrease in responsiveness (desensitization) to a drug, taking days or weeks to develop. Unlike tachyphylaxis, tolerance cannot be demonstrated *in vitro* experimental situations. Additionally, in tachyphylaxis even an increased dose does not elicit the pharmacological response, while in tolerance an increased dose may elicit a maximal response. Multiple mechanisms may account for the development of tolerance such as induction of microsomal enzymes (e.g. barbiturates), activation of homeostatic (compensatory) mechanisms (e.g. vasodilator antihypertensives) and down regulation of receptors (e.g. opioid analgesics).

Drug resistance: The term drug resistance is used to describe the loss of effectiveness of antimicrobial drugs against microbes. This primarily occurs due to development of resistance in microorganisms to certain antimicrobial drugs (see for detail, Chapter 58).

Reverse tolerance: Reverse tolerance or sensitization is the phenomenon of a reversal (suppression) of the side-effects from a drug, or the reduction of insensitivity caused after drug tolerance has been established. Typically, the reverse tolerance occurs after the use of an additional drug, or abstinence from a drug for a period of time, known as a drug holiday. Reverse tolerance is seen with drugs like amfetamines and selective serotonin receptor inhibitors (SSRIs). As a result of reverse tolerance, regular administration of some drugs produces a quick decrease of unwanted side effects, without an equivalent loss of desirable properties. The sensitization is induced more quickly and persists far longer than withdrawal-related effects.

Selectivity, Specificity and Sensitivity

Selectivity: Selectivity is the capacity or propensity of a drug to affect one receptor or cell population in preference to others. In other words, selectivity suggests that a drug in normal doses acts only on one type or sub-type of a receptor and produces its pharmacological effect. Selectivity can be measured or described by means of such indices as the therapeutic index or the standardized safety margin. Selectivity is generally a desirable property in a drug e.g. it is desirable that an anti-adrenergic drug metoprolol selectively act on β_1-adrenergic receptor in heart and produces positive inotropic effect. However, sometimes selectivity of action for receptors or cells is not necessarily desirable and a non-selective drug is preferred, as in the treatment of organophosphorus insecticide poisoning a non-selective antimuscarinic drug atropine is used due to involvement of all muscarinic receptor subtypes.

Specificity: Specificity is the capacity of a drug to manifest only one kind of action. A drug of perfect specificity of action might increase or decrease a specific function of a given cell type, but it would not do both. For example, acetylcholine is *non-specific* in its action as it stimulates exocrine glands, depresses myocardial cells and contracts smooth muscles about the same dose rate. Similarly nicotine is also not specific in its actions in autonomic ganglia as first it stimulates and then depresses ganglionic functions by a number of means. In contrast to above drugs, warfarin is very specific in action as it blocks only the coagulation of blood.

Selectivity and specificity are frequently used as synonyms for each other, but actually they describe separate phenomena, and must not be confused. Selectivity is concerned with the site of action and specificity with the kind of action at a site.

Sensitivity: Sensitivity is the ability of a population, an individual, a tissue or a receptor, relative to the abilities of others, to respond in a qualitatively normal fashion to a particular drug dose. The smaller the dose required for producing an effect, the more sensitive is the responding system. An animal would be considered abnormally sensitive to morphine if a small fraction of the normal opioid dose gives adequate pain relief; or insensitive to morphine if an abnormally large dose of analgesic provides pain relief. If an animal manifests an allergic response after taking morphine, then it would be considered hypersensitive to the opioid, regardless of whether the analgesic provides relief from pain. Sensitivity may be measured or described quantitatively in terms of the point of intersection of a dose-response curve with the axis of abscissal values. In other words, the sensitivity of a measuring system is defined as the lowest input (smallest dose) required to produce a given degree of output (effect).

Terms Related to Receptors

Spare receptors/Reserve receptors: These are the receptors, which remain unoccupied (free) during elicitation of the pharmacological response by an agonist. It has been estimated that highly active drugs (e.g. epinephrine and histamine) require only a small fraction of receptors (as low as < 1%) to produce their maximal response. In such situations, a large number of receptors remain unoccupied at therapeutic concentrations, and the system may be said to possess 'spare receptors' or a 'receptor reserve'.

Silent receptors: These are the receptors which do not elicit a pharmacological response even when they are occupied by an agonist. The term is less used because such receptor sites mainly serve as drug acceptors or binding sites. It is suggested that presence of silent receptors or drug acceptors usually prolong the action of drugs in body as the receptor bound drugs are slowly released from such receptors and are then available for the active receptors.

Orphan receptors: These are the receptors those have similar structures to other identified receptors but for them the endogenous ligand has not yet been identified or synthesized. These types of receptor are generally synthesised by cloning of a conserved segment of DNA molecule (that encodes for a receptor) and are utilized for search of a new endogenous ligand or drug. If a ligand for an orphan receptor is later discovered, the receptor is referred to as an adopted orphan receptor. Examples of orphan receptors are found in the G protein-coupled receptor (GPCR) and nuclear receptor superfamilies. GPCR orphan receptors are usually given the name "GPR" followed by a number (e.g. GPR1).

Putative receptors: These are ligand-binding sites, which are suspected to function as receptors, but as receptors they have not been fully characterised.

DOSE-RESPONSE RELATIONSHIP

The relationship between the dose of a drug and the magnitude of response (effect) it produces is called the dose-response relationship (in case of concentration, the term concentration-

response relationship may be used). Usually by increasing or decreasing the dose, the response also increases or decreases, respectively and such a relationship becomes the dose-response relationship. If such data are plotted on a graph, then the curve obtained is called dose- response curve.

Types of Dose-Response Relationship

The dose-response relationship is broadly of two types: graded and quantal dose-response relationships.

1. Graded/Gradual Dose-Response Relationship

The graded or gradual response is one involving a continual change in the effect with changing dosages i.e. the response increases with increase in dose and decreases with decrease in dose. Most drug responses fall in this category. The graded dose-response relationship is useful in measuring the incremental responses of drugs and can be seen in an individual organism e.g. contraction or relaxation of smooth muscles, change in blood pressure, intensity of adverse effects, etc. This type of relationship is useful in studying the efficacy of therapeutic drugs or toxic symptoms with toxicants.

The graded dose-response relationship is based on following presumptions:

a. The pharmacological effect is a result of the known drug.

b. There is a molecular or receptor site(s) with which the drug interacts to produce the response.

c. The production of a response and the degree of response are related to the concentration of the drug at molecular or receptor site.

d. The concentration of drug at the molecular or receptor site, in turn, is related to the administered dose of the agent.

e. The effect of drug is proportional to the fractions of molecular or receptor site occupied by the agent; therefore by increasing or decreasing the dose, the response also increases or decreases, respectively. The maximal effect occurs when the drug occupies all molecular or receptor sites.

When a graded response versus dose is plotted on a simple graph paper, the dose-response curve assumes a shape of parabola with its origin at zero on both axes. In case of response versus log-dose, the curve becomes sigmoid (S-shaped) instead of hyperbolic (Figure 6.10). The logarithmic transformation of dosage is commonly used for plotting dose-response relationship because it provides more linear representation of data, permits wide range of doses on a single graph and facilitates visual and mathematical comparisons between dose-response curves for different agents or for different responses to a single agent.

From a graded dose-response curve, some valuable parameters can be obtained like:

a. ***Threshold dose:*** It is the minimal dose that produces the desired observable/recordable pharmacological response.

b. ***Ceiling dose/Effective dose-100 (ED_{100}):*** It is the minimal dose that produces the maximal (100%) response (E_{max}). Beyond the ceiling dose, there will be no increase in response even if the dose is increased many fold because no more spare receptors are available to the drug.

c. ***Maximal response/Maximal effect (E_{max})/Ceiling effect:*** It is the maximal response produced by a drug under specified conditions. Capability to produce the maximal response indicates maximal efficacy, and is a characteristic of the drug.

d. ***Effective dose-50 (ED_{50})/Median effective dose (MED):*** It is the dose that produces half (50%) of the maximal response.

2. Quantal / All-or-None Dose–Response Relationship

Quantal dose-response relationship is one involving an all-or-none response i.e. on increasing the dose of a drug, the response is either produced or not. This type of relationship is seen with certain responses which follow all or none phenomenon and cannot be graded e.g. hypnosis, blood coagulation and death. The graph of a quantal dose-response relationship does not show the intensity of effect, but rather the frequency with which any dose produces the all-or-none phenomenon. The quantal dose-response relationship is always seen in a population because the assumption is made that individuals respond to maximal possible or not at all.

In quantal dose-response, the log-dose response curve is sigmoid in character (Figure 6.10). The sigmoid curve has a relatively linear portion between 16 and 84 per cent, which is used to determine the slope of the curve. A small proportion of population at left and right sides of the curve respond to low and high doses and constitute hyper-reactive (hypersensitive) and hypo-reactive (hypo-sensitive) groups, respectively (If a drug produces its usual effect at very low dosage, the individual is said to be hyper-reactive or hypersensitive; if the same response is produced by the drug at unusually large dosage, the individual is said to be hypo-reactive or hyposensitive). To make the relationship linear, the responses are frequently converted into probits (probability units) from statistical table, and log-dose probit response curve is plotted (Figure 6.10). This linear curve gives the slope of dose-response curve more accurately, allows easy comparison of two curves and is useful for calculating different lethal/effective doses (e.g. LD_{10},

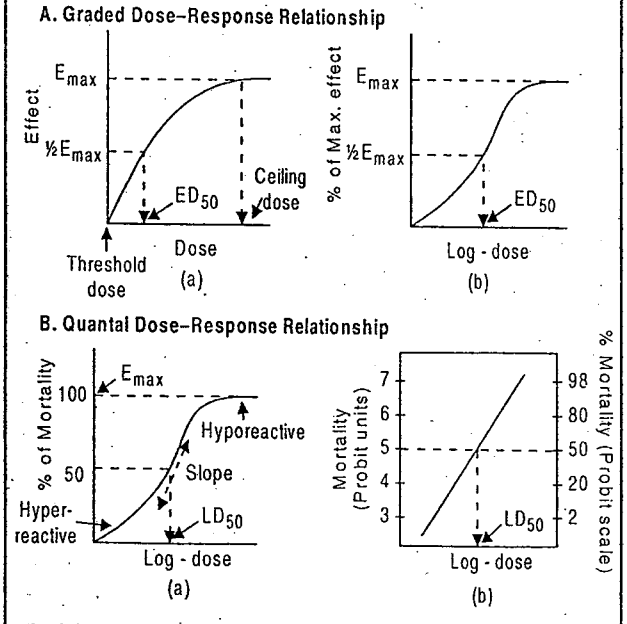


Fig. 6.10. Different representation of dose-response curve.
A: Graded-dose-response relationship (a) Hyperbolic curve (dose-response plot) (b) Sigmoid curve (log-dose-response plot).
B: Quantal dose-response relationship. (a) Sigmoid curve (log dose-response plot) (b) Linear curve (log dose-probit response plot).

LD_{50}, LD_{90}, etc.). The quantal dose-response curve is extensively used in toxicology for calculation of lethal dose-50% (LD_{50}). In case of non-lethal responses, ED_{50} can be similarly determined. The curve can also be used to determine a specific toxicity parameter like median toxic dose or toxic dose-50 (TD_{50}) i.e. a dose which produces toxicity in 50% of the animals in a population.

Lethal dose-50 (LD_{50}) / Median lethal dose (MLD): Lethal dose-50 (LD_{50}), also called median lethal dose (MLD), is the dose that is lethal to 50 per cent of animals exposed to a given toxicant under defined conditions. The LD_{50} value does not pertain to the severity of clinical signs observed or the characteristic changes caused by the toxicant but depends only on the lethality produced by a toxicant. Though recently some toxicological organisations and government regulatory agencies have greatly reduced reliance on the LD_{50} in order to reduce the number of animals needed for study, yet it is still considered an important index to assess the toxicity of chemicals.

The mid-point of the graded or quantal dose response curve (ED_{50} or LD_{50}) is usually taken for most calculations because it is a convenient description of average response. The majority of animals responding to a drug do so symmetrically about the mid-point. Further there is least variability at 50% level of response (i.e. 95% confidence limits are narrowest at ED_{50} or LD_{50} point).

Variables of Dose-Response Curve

Irrespective of the shape (hyperbolic, sigmoid or linear), the dose-response curve has 4 characteristic variables: efficacy, potency, slope and variance.

1. Efficacy

Efficacy (Maximal efficacy, E_{max}) is the maximal effect or response produced by a drug. Efficacy of a drug may be determined by its inherent properties or those of the receptor-effector system, and is indicated by the height (maximal response possible for the drug) of the dose-response curve. Efficacy is independent of the slope or position (potency) of dose-response curve. A full agonist, which produces maximal response, has maximal efficacy. A partial agonist that fails to produce maximal response irrespective of its dose has low or poor efficacy and an antagonist that does not produce pharmacological response has zero efficacy (Table 6.3). Efficacy of a drug is generally considered more important than its potency in selecting drugs for a clinical situation because it determines the intensity or magnitude of response produced by a drug.

In Figure (6.11a), drug A and drug B have equal efficacy because they produce same maximal effect (E_{max}). However in Figure 6.11b, the drug A produces more response so is more efficacious than the drug C. For example, morphine and aspirin are both analgesics, but they have different efficacies. The morphine provides relief from pain of nearly all intensities, whereas aspirin is effective against only mild to moderate pain. Pethidine, a morphine substitute, is almost equally efficacious analgesic as morphine.

2. Potency

Potency is a measure of drug activity expressed in terms of the amount or dose required to produce an effect of given intensity. Potency of a drug depends on its affinity for the receptor or target site and efficiency of drug-receptor interaction. The position of dose-response curve on dose axis is taken as index of the drug potency and the parameter that determines potency is the ED_{50} or LD_{50}. Accordingly, a drug producing the desired effect in a smaller dose is called more potent than another drug producing the same effect at a higher dose. In Figure 6.11a, drug A is more potent than drug B, since the dose of drug A is smaller than the dose of drug B to produce the given effect (i.e. ED_{50}). For example, pethidine is less potent but equally efficacious analgesic as morphine i.e. both can remove pain of all intensities, but only at different doses.

Comparison of potency between two drugs is meaningful only when the agonists being compared have dose-response curves of the same slope. Potency is independent of the efficacy and is considered important for selecting the drug dosage and not the clinical action. Therefore, potency is not a very important property of drug, provided the formulated form of the drug can be conveniently administered (i.e. dose should neither be too small nor too large). In toxicology, however, extremely potent drugs require special handling due to their low LD_{50} values. For therapeutic applications, the potency of a drug is necessarily stated in absolute dosage units (100 mg, 10 mg/kg body weight, etc.), but for comparison of drugs, relative potency or ratio of equieffective doses (½ x, 5 x, etc.) are conveniently used.

Often the terms, drug potency and drug efficacy are used interchangeably, but they are not synonyms. Both refer to different characteristics of a drug and should not be confused.

3. Slope

The slope of a dose-response curve reflects the binding of a drug to its receptor and thus the affinity between the receptor site and the drug. Steep slope of dose-response curve indicates more binding (affinity) of drug with its receptor and suggests that only slight increase in dose will markedly increase the pharmacological response. On the other hand, a flat curve indicates lesser binding between drug and receptor, suggesting that a wide range of doses produces only a little increase in response. In a population, the slope indicates the range of doses over which the population is affected. A steep slope indicates that a large proportion of population will be affected over a small range of doses and a shallow dose-response curve suggests that wide range of doses are required to produce the desired response in a population. Steeper curve also suggests that response of a compound in a population is more uniform and more predictable. The shallow curve, in contrast, reflects non-uniform response of a drug in a population, which is difficult to predict due to greater variations in the response. A shallow curve also puts hypersensitive individuals to greater risk than general population.

In therapeutics, slope carries less importance except for drugs which have low margin of safety. Toxic drugs those have steep dose-response curves are potentially more difficult to use because

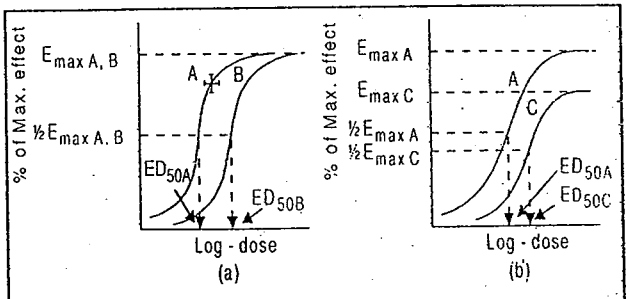


Fig. 6.11. Comparison of dose-response curves of two drugs :
(a) Two drugs having same efficacy but different potency,
(b) Two drugs having different efficacy.

only a small increase in dose may produce toxicity. For example, a steep dose-response curve for a barbiturate (e.g. pentobarbital) implies that there is a small ratio between the dose that produces sedation/hypnosis and that which causes death. In toxicology, slope of a dose-response curve of a compound should neither be too steep nor too shallow. In considering lethality, slope of curve also facilitates comparison of toxicities of two agents.

4. Variance

Variance (variation, biological variation) can be defined as the appearance of differences in magnitude of response among individuals in same population given same dose of a drug. The intersecting brackets in Figure 6.11a indicate that biological variations of the dose-response relationship can be visualised in either of the two ways. The vertical bracket expresses the fact that a range of effects will be produced by a given dose in a population. Alternately, the horizontal bracket expresses the fact that a range of doses is required to produce a specified intensity of effect in all individuals.

Measures of Safety

The safety of drugs is often given by comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes death.

1. Therapeutic index

Therapeutic index may be defined as the ratio between the lethal dose-50 (LD_{50}) and the effective dose-50 (ED_{50}).

$$\text{Therapeutic index (T.I.)} = \frac{LD_{50}}{ED_{50}}$$

Where, LD_{50} is the dose that is lethal for 50% of the population and ED_{50} is the dose that is effective for 50% of the population. Therapeutic index is widely used for evaluating the safety and usefulness of a drug. The higher the therapeutic index, the safer is the drug. The therapeutic index value of a drug also gives a rough idea of drug's selectivity. A high value indicates that drug is highly selective in its action, while a low value indicates that the drug is non-selective in action. In therapeutics, a drug with high therapeutic index is preferable and widely used in comparison to that with a low one, which requires special and careful handling. For example, diazepam with a therapeutic index of about 100 is widely and safely used, while general anaesthetics with index less than 2 require careful monitoring during administration. Other examples of drugs with a narrow therapeutic range, that may require drug monitoring to achieve therapeutic levels and minimize toxicity, include digoxin, dimercaprol, warfarin and lithium carbonate.

The therapeutic index is only valid for comparison if the therapeutic dose-response curve (ED_{50} curve) and the lethality dose-response curve (LD_{50} curve) are parallel (Figure 6.12a). If the two curves are not parallel e.g. if dose-response curve for lethality is shallower than that for therapeutic curve (Figure 6.12b), then there will be decreasing safety at lower doses and the hypersensitive groups will be at higher risk. Here, the drug will be much more toxic at 1% or 10% levels, and the therapeutic index would be misleading. In such a case (when curves are not parallel), therapeutic ratio or certain safety factor may be used. In clinical situations also therapeutic index has certain limitations like a therapeutic agent may have more than one ED_{50} values, one for each of its desired effects; therefore, there may be more than one therapeutic index. In therapeutics, toxic symptoms are more relevant than lethality; therefore, the protective index is sometimes taken as a better safety measure.

2. Therapeutic ratio

Therapeutic ratio may be defined as the ratio between the lethal dose-25 (LD_{25}) and the effective dose-75 (ED_{75}).

$$\text{Therapeutic ratio (T.R.)} = \frac{LD_{25}}{ED_{75}}$$

Where, LD_{25} is the dose that is lethal for 25% of the population and ED_{75} is the dose that is effective for 75% of the population. Therapeutic ratio is considered a better index of safety of a drug as it includes slope of curve also. Shallow dose-response curves usually have low therapeutic ratios. A flat curve is considered more toxic because the hypersensitive groups are at much more risk than the hypo-sensitive or normal groups. In Figure 6.13, computation of the therapeutic ratio values reveals that compound B is more toxic than compound A as the

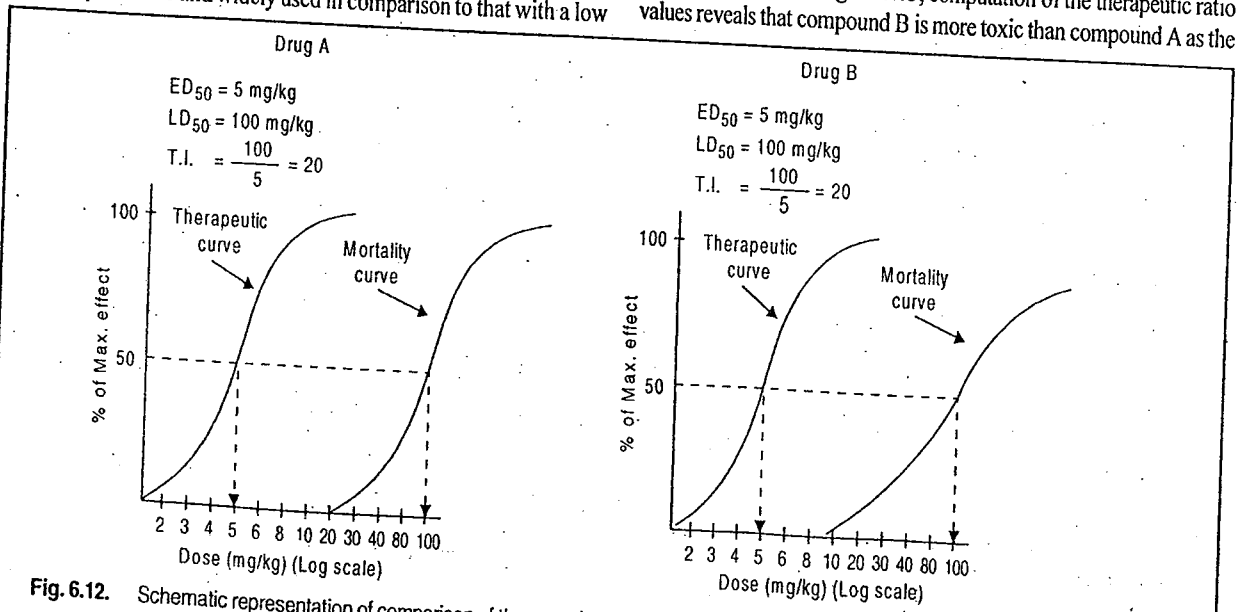


Fig. 6.12. Schematic representation of comparison of therapeutic index of two drugs (see text for detail).
Drug A : Therapeutic and mortality curves are parallel. Drug B : Therapeutic and mortality curves are not parallel.

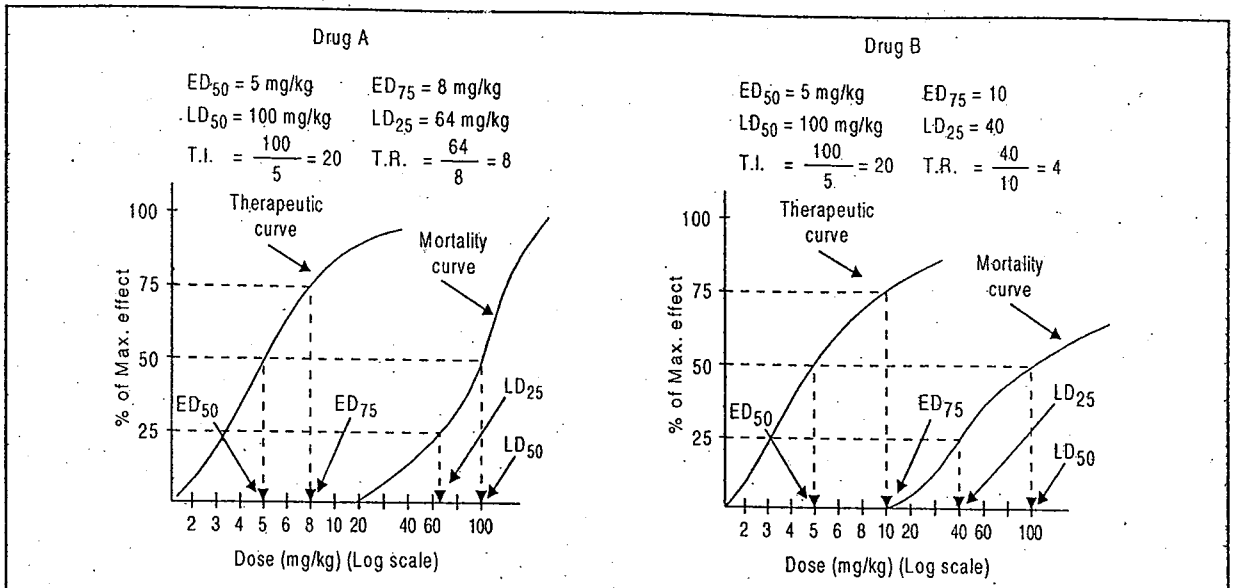


Fig. 6.13. Schematic representation of therapeutic ratios of two drugs. (See text for detail) (Graduations are not to scale).
Drug A : Therapeutic and mortality curves are not parallel but steeper than those of drug B.
Drug B : Therapeutic and mortality curves are not parallel but flatter than those of drug A.

compound B has a narrow therapeutic ratio due to a flatter curve. Both drugs, however, have the same therapeutic index.

3. Certain safety factor

Certain safety factor may be defined as the ratio between the lethal dose-1 (LD_1) and the effective dose-99 (ED_{99}).

$$\text{Certain safety factor (CSF)} = \frac{LD_1}{ED_{99}}$$

Certain safety factor is a more convenient estimate of safety than the therapeutic index as the values are derived from extremes of the respective dose-response curves. Unlike the therapeutic index, the meaningfulness of the certain safety factor does not depend on the parallelism of the dose response curves from which the LD_1 and ED_{99} are inferred. However, values of minimum toxic (LD_1) and maximum therapeutic (ED_{99}) are less precise than the median doses (LD_{50} and ED_{50}), and difficulty of determining such values with accuracy excludes this possibility (In quantal dose-response relationship, a normally distributed curve approaches a response of 0 per cent as the dose is decreased and approaches 100 per cent as the dose in increased, but theoretically it never passes through 0 and 100 percent values, so 1% and 99% values are taken as minimum and maximum response, respectively).

4. Standardized safety margin

Standardized safety margin (SSM) is given by a value LD1-ED99/ED99 x 100%, and to some extent similar to certain safety factor. It indicates by what percentage of itself a dose effective in virtually all (99%) of a population must be exceeded in order to produce a lethal effect in a minimum number (1%) in the population. Clinically, the standardized safety margin probably has greater practical meaning than does the therapeutic index, but it suffers from the same limitations as mentioned for certain safety factor.

5. Protective index

A protective index may be defined as the ratio between the toxic dose-50 (TD_{50}) and the effective dose-50 (ED_{50}).

$$\text{Protective index (P.I.)} = \frac{TD_{50}}{ED_{50}}$$

Where, TD_{50} is the dose that is toxic for 50% of the population and ED_{50} is the dose that is effective for 50% of the population. The protective index is similar to the therapeutic index, but concerns toxicity (TD_{50}) rather than lethality (LD_{50}); thus, the protective index is a smaller ratio. The protective index is useful in clinical conditions where a side effect or an adverse (toxic) effect is taken into considerations and not the lethality. Similar to therapeutic index, a high protective index is preferable to a low one. A drug should ordinarily only be administered if the protective index is sufficiently greater (at least more than 1), indicating that the benefit outweighs the risk. The protective index is a more accurate measure of the benefit-to-risk ratio than the therapeutic index, but is less objectively defined.

6. Risk-benefit ratio

Risk-benefit ratio is a term used to indicate a judgement on the estimated harm (e.g. adverse effect, cost, etc.) versus expected advantages (e.g. cure, relief of symptoms, etc.). Risk-benefit ratio cannot be measured accurately, but is based entirely on the data collected from use of drugs in large population and on the clinician's own experience of the drug and the patient. A drug should be prescribed only when the benefits outweigh the risks.

DRUG INTERACTIONS

A drug interaction is a situation in which a substance affects the activity of a drug i.e. the effect is either increased or decreased. The interactions may occur between two drugs (drug-drug interaction), between drug and food (drug-food interaction), as well as drug and herbs (drug-herb interaction). However, in practice when the term drug interaction is used it invariably means interaction between two or more drugs given together or in quick succession. The drug interaction is a very important phenomenon and affects the outcome of a therapy. In most cases, the drug interactions occur out of accidental misuse of drugs or due to lack of knowledge about the pharmacology of active ingredients. In some cases it is deliberate to get a desirable pharmacological response.

Table 6.4: Important pharmacokinetic and pharmacodynamic drug interactions.

Drug(s) affected (First drug)	Modifying drug(s) (Second drug)	Consequence and effect of interaction on first drug*
I. Pharmacokinetic drug-interaction		
Absorption		
a. Tetracyclines, ciprofloxacin	Antacids (containing Ca, Mg, or Al salts), iron preparations.	Reduced absorption due to complex formation.
b. Ketoconazole	H_2-blockers (e.g. cimetidine ranitidine), omeprazole.	Reduced absorption due to increased gastric pH.
c. Rifampicin, lincomycin	Kaolin.	Reduced absorption due to adsorption.
d. Vitamins	Liquid paraffin.	Reduced absorption due to GI mucosal coating.
e. Procaine	Epinephrine.	Reduced absorption due to localised vasoconstriction.
Distribution		
a. Sulphonamides, warfarin	Phenylbutazone, salicylates.	Enhanced concentration due to displacement from plasma protein binding sites.
b. Iron	Deferoxamine.	Reduced concentration due to chelation.
c. Digoxin	Quinidine, verapamil, amiodarone.	Enhanced concentration due to displacement from tissue binding sites.
Biotransformation		
a. Warfarin, corticosteroids	Phenobarbital, rifampicin, griseofulvin, phenytoin.	Enhanced metabolism due to microsomal enzyme induction.
b. Tolbutamide, pentobarbital, phenytoin	Chloramphenicol, cimetidine.	Reduced metabolism due to microsomal enzyme inhibition.
c. Pethidine, epinephrine, tyramine	MAO-Inhibitors (e.g. tranylcypromine).	Reduced metabolism due to monoamine oxidase (MAO) enzyme inhibition.
Excretion		
a. Amfetamine, quinidine	Ammonium chloride.	Enhanced renal excretion due to acidification of urine (reduced urine pH).
b. Salicylates, barbiturates	Sodium bicarbonate, potassium citrate.	Enhanced excretion due to alkalinisation of urine (increased urine pH).
c. Penicillins, cephalosporins	Probenecid.	Reduced urinary excretion due to competitive inhibition of tubular carrier system.
II. Pharmacodynamic drug interactions		
Receptor mediated		
1. Action on same receptor		
a. Morphine and related opioids	Naloxone	Decreased action due to receptor antagonism.
b. Isoprenaline, terbutaline	Propranolol	Decreased action due to receptor antagonism.
c. Epinephrine	Norepinephrine	Enhanced (additive) action on blood vessels and heart.
2. Action on different receptors		
a. Epinephrine	Acetylcholine	Reduced (antagonistic) action on blood vessels and heart.
b. Terbutaline, clenbuterol	Atropine	Enhanced bronchodilation.
Non-receptor mediated		
a. Penicillin G	Streptomycin	Enhanced (synergistic) antibacterial action.
b. Sulphonamides	Trimethoprim	Enhanced (synergistic) antibacterial action.
c. Warfarin	Aspirin	Enhanced bleeding.

* Consequences and effects of drug interactions have been shown mainly on the first drug. However, in many examples, pharmacological effect of second drug may also be similarly modified.

Mechanisms of Drug Interaction

Drug interactions are known to occur by a number of mechanisms including alterations in the pharmacokinetics or pharmacodynamics of one or both of the interacting drugs. The interaction in which one drug alters the pharmacokinetics (absorption, distribution, biotransformation and excretion) of second drug, thereby affecting its concentration and response in body, is called the pharmacokinetic interactions (Table 6.4). Interaction in which there is no alteration in the pharmacokinetics but a change in the biological action at the target site is called the pharmacodynamic interaction. Pharmacodynamic interaction generally occurs due to involvement of either receptor or non-receptor mediated events. In receptor mediated interactions, two or more drugs act on one or more types of receptor to produce interactions. Non-receptor pharmacodynamic interactions occur when two or more drugs having different mechanisms of action act on certain non-receptor target sites (e.g. enzymes, carrier proteins, ion-channels, physiological cascade systems, etc.). Some important pharmacokinetic and pharmacodynamic drug interactions are enlisted in Table 6.4

Types of Drug Interaction

When two or more drugs are used together, the pharmacological response is not necessarily the sum of two agents used individually. Depending on the magnitude of pharmacological response produced by the combination of two drugs (irrespective of mechanisms), drug interactions are broadly divided into 4 types: addition, potentiation, synergism and antagonism.

1. Addition

When the combined effect of two drugs given together is equal in magnitude to sum of the effects of each drug given alone, the drug interaction is called addition and the effect produced is called additive effect.

$$E_{AB} = E_A + E_B \text{ or } 1 + 1 = 2$$

Where, E_{AB} = Combined effect of two drugs A and B.
E_A = Effect of Drug A alone.
E_B = Effect of drug B alone.

The additive effect is produced mainly when two drugs administered together have same/similar mechanism of action and their effects are additive. For example, combination of two organophosphorus insecticides produces additive cholinesterase inhibition and combination of two or more sulphonamides (sulphadiazine + sulphamerazine + sulphamethazine) produces additive antibacterial effect.

2. Potentiation

When one drug having no effect of its own increases the pharmacological effect of another drug, the interaction is called potentiation and the effect produced is called potentiative effect.

$$E_{AB} > E_A + E_B \text{ or } 1 + 0 > 1$$

The potentiative effect is seen mainly when one drug having no effect of its own enhances the concentration of other drug at its site of action either by increasing its absorption or by decreasing its elimination. For examples, carbidopa (dopa-decarboxylase inhibitor) enhances central effect of levodopa by decreasing its peripheral metabolism, probenecid enhances effect of penicillins by decreasing their renal excretion, clavulanic acid increases antibacterial efficacy of amoxicillin by causing inhibition of beta-lactamase enzymes, and MAO inhibitors (e.g. tranylcypromine) enhance antihypertensive effect of catecholamines.

3. Synergism

When the combined effect of two drugs is greater in magnitude than sum of the effects of each drug given alone, the interaction is called synergism and the effect produced is called synergistic effect.

$$E_{AB} > E_A + E_B \text{ or } 1 + 1 > 2$$

The synergistic effect is produced mainly when two drugs used together have same or similar effects and they increase each other's action at the site of action. For example, both carbon tetrachloride and ethanol are hepatotoxicants, but together they produce much more injury to the liver than expected from the mathematical sum of their individual effects. Similarly, individually both sulphamethoxazole and trimethoprim are bacteriostatic agents, but their combination produces synergistic bactericidal effect.

4. Antagonism

When the combined effect of two drugs is lesser in magnitude than sum of the effects of each drug given alone, or when one drug having no effect of its own decreases or inhibits the pharmacological effect of other drug, the interaction is called antagonism and the effect produced is called antagonistic effect.

(*i*) $E_{AB} < E_A + E_B \text{ or } 2 + 1 < 3$

(*ii*) $E_{AB} < E_A \text{ or } 2 + 0 < 2$ (True antagonism)

The antagonistic effect is produced mainly when a partial agonist is combined with a full agonist (i) or when an antagonist and an agonist are combined together (ii, true antagonism). Examples of true antagonism include combination of morphine (opioid receptor agonist) and naloxone (opioid receptor antagonist), and isoprenaline (β-adrenoceptor agonist) and propranolol (β-adrenoceptor antagonist).

ANTAGONISM

Antagonism (Greek word *antagonisma*, from anti = against, *nizomai* = to fight) is an important drug interaction, which decreases the usefulness of a prescription. However, antagonistic effects of drugs are often very desirable in therapeutics and are basis of the action of many antidotes.

Types of antagonism

Drug antagonisms are broadly of four types: chemical, functional, dispositional and receptor-mediated antagonisms.

1. Chemical antagonism

Chemical antagonism (inactivation antagonism) occurs when two or more drugs react chemically and form an inactive product. This type of antagonism occurs primarily when two agents have opposite chemical properties, which neutralize each other i.e. acid and base, cations and anions. For examples, sodium bicarbonate neutralises acidic compounds and protamine (basic and cationic drug) neutralises anticoagulant effect of heparin (acidic and anionic drug), and 5% dextrose saline (acid pH) inactivates the acid-susceptible antibiotic penicillin G. Chemical inactivation also occurs when dimercaprol (BAL) chelates metal ions such as arsenic, mercury and lead and decreases their toxic effects.

2. Functional antagonism

Functional antagonism (Physiological antagonism) occurs when two drugs counterbalance (neutralise) each other by producing opposite effects on the same physiological system. Here two drugs are acting on two different systems or receptors and

produce pharmacological response in opposite directions. For example, acetylcholine and epinephrine act on muscarinic and adrenergic receptors on heart to produce bradycardia and tachycardia, respectively. In clinical practice, fall in blood pressure produced by severe barbiturate intoxication can be effectively antagonised by vasopressor agents like norepinephrine or metaraminol, and strychnine induced convulsions can be controlled by systemic administration of CNS depressants like pentobarbital sodium. Other examples of physiological antagonism include effects of insulin and glucagon on blood sugar level, and hydrochlorothiazide and amiloride on urinary K^+ execration.

3. Dispositional antagonism

Dispositional antagonism (pharmacokinetic antagonism) occurs when the disposition or pharmacokinetics (i.e. absorption, distribution, metabolism and excretion) of a drug is altered so that concentration and/or duration of the drug at the target site is diminished. Dispositional antagonism is very common in therapeutics. For example, antacids enhance dissolution and absorption rate of sulphonamides, salicylates enhance toxicity of warfarin due to its displacement from the plasma protein binding sites, and phenobarbital decreases plasma levels of corticosteroids by enhancing their metabolism through induction of microsomal enzymes (Table 6.4).

4. Receptor antagonism

Receptor antagonism (Pharmacological antagonism) occurs when two drugs act on the same receptor to produce less of an effect. Agonist increases the effect and antagonist decreases or abolishes it. Both agonist and antagonist are specific to the receptor. Receptor antagonism is primarily of two subtypes: competitive and non-competitive antagonisms.

*i. **Competitive antagonism:*** In competitive (reversible, surmountable) antagonism, the antagonist and agonist compete with each other for the occupancy of same binding site (active site) on the receptor. The interaction between the drug (agonist or antagonist) and the receptor is due to formation of weak bonds, so the effects of one drug can be easily overcome by increasing the concentration of second drug. Both agonist and antagonist have affinity for the same receptor, but drug in higher concentration occupies the receptor. Often (though not always) the antagonists possess a similar chemical structure to that of the agonist.

In dose-response relationship, the competitive antagonist causes a parallel shift of the log dose-response curve to the right, but there is no change in its shape, slope or maximal response. In Figure 6.14, the potency (affinity) of agonist decreases in presence of antagonist, but efficacy (E_{max}) remains the same as it can be achieved at a higher dose. In Line-Weaver Burk or double reciprocal plot, the lines meet at 1/E axis, which gives the same E_{max}. The lines diverge at higher agonist concentration indicating that apparent affinity of agonist for the receptor is lowered (reciprocal of dissociation constant i.e. $1/K_D$ is affinity constant). Competitive receptor antagonism is seen when atropine blocks action of acetylcholine on muscarinic receptors, propranolol blocks action of isoprenaline on β-adrenoceptors, and naloxone blocks action of morphine on opioid receptors.

*ii. **Non-competitive antagonism:*** In non-competitive (irreversible, un-surmountable) antagonism, the antagonist forms strong bonds (e.g. covalent bond) with its receptor and irreversibly blocks it. Therefore, antagonist cannot be displaced by the agonist, even when its high concentration is used. The non-competitive antagonists generally bind to a distinctly separate binding site (allosteric site) from the agonist, and may prevent conformational changes in the receptor required for receptor activation after the agonist binds. The agonist can now act only on reserve receptors or receptor sites not influenced by the antagonist. The affinity of the agonist for receptor is thus unaltered but its efficacy is reduced, as it cannot produce the maximal response (E_{max}) irrespective of its dose.

The log dose-response relationship shows flatter curves with reduced efficacy (Figure 6.15). The double reciprocal plot shows meeting of lines at $-1/K_D$ on the 1/dose axis. The plot shows unaltered affinity ($-1/K_D$ is same) but decreased efficacy (altered $1/E_{max}$). Phenoxybenzamine has been shown to act as a non-competitive antagonist of epinephrine at the α-adrenoceptors. Interaction of phenoxybenzamine with α-adrenoceptors is reversible initially but after some time it becomes irreversible due to formation of strong bonds between them. Non-competitive antagonisms are also seen between diazepam and bicuculline, and acetylcholine and decamethonium.

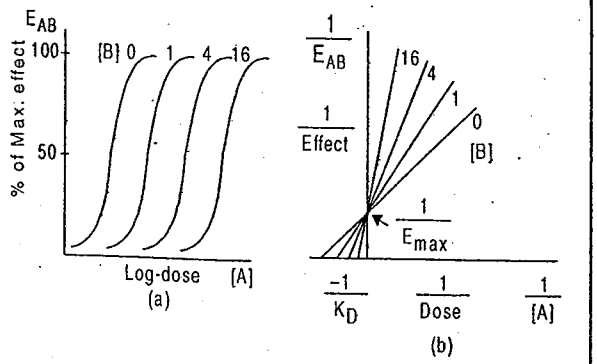


Fig. 6.14. Dose-response curves depicting competitive antagonism between agonist (A) and antagonist (B) (See text for detail). (a) Sigmoid curves, (b) Double reciprocal plot.

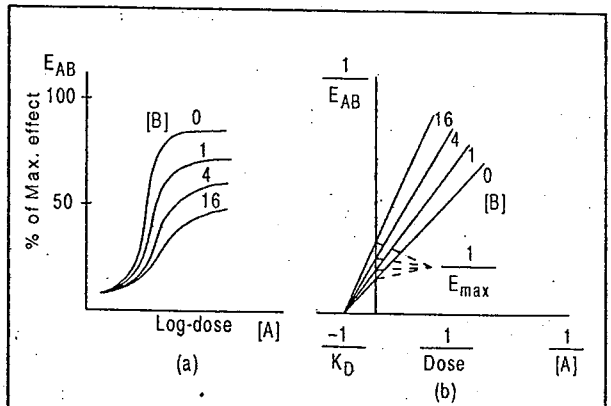


Fig. 6.15. Dose-response curves depicting non-competitive antagonism between agonist (A) and antagonist (B). (a) Sigmoid curves, (b) Double reciprocal plot.

Table 6.5: Comparative features of competitive and non-competitive antagonisms.

Features	Competitive antagonism	Non-competitive antagonism
Receptor binding	Reversible (van der Waals or hydrogen bonds).	Irreversible (covalent bonds).
Site of receptor binding	Antagonist binds with the same receptor site as the agonist.	Antagonist may either bind with the same receptor site as the agonist or bind to an allosteric site (site different from agonist binding site).
Chemical structure of antagonist	Antagonist usually resembles in structure with agonist.	Antagonist may or may not resemble in structure with agonist.
Agonist-antagonist interaction	Competitive	Non-competitive
Dose-response curve		
a. Efficacy (E_{max})	Remains same	Decreases
b. Slope	Remains same	Decreases
c. Position	Shifts to right	Remains same
Intensity of response	Depends on concentration of both agonist and antagonist.	Depends only on the concentration of antagonist.
Antagonism	Surmountable	Un-surmountable.
Duration of action	Short, depends on drug clearance.	Long, depends on new receptor synthesis.
Examples	Acetylcholine-atropine Morphine-naloxone	Norepinephrine-phenoxybenzamine. Diazepam-bicuculline.

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CHAPTER 7 FACTORS MODIFYING DRUG EFFECT AND DOSE

Factors related to drug
Factors related to exposure conditions
Factors related to subject
Factors related to environmental conditions

Considerable variations in response to the same drug between species, breeds, and individuals and even in the same individual at different occasions is observed. These variations in drug response are attributable to numerous unintended variables called factors, which influence drug action qualitatively and/or quantitatively. These factors can be broadly divided into 4 groups: factors related to drug, exposure conditions, subject, and environmental conditions.

I. Factors Related to Drug

1. Nature and types of dosage form

The response of a drug may vary with the dosage form of that drug. The nature and types of the dosage form may alter the rate of absorption and bioavailability of the drug to about 2 to 5 fold or even more thus altering the pharmacological response and dosage. Compared with solid dosage forms, liquids are absorbed faster and generally cause rapid onset of action because disintegration and dissolution are not a concern with solutions, as it may be for solids. As a general rule, absorption and action of drugs are fastest from solutions and slowest from depot and sustained release products i.e. solutions > emulsions > suspensions > capsules > tablets > sustained released products.

2. Types of salt form

Most drugs are weak electrolytes (acids or bases) and are available commercially in their salt forms. Generally with weak acidic drugs (e.g. barbiturates and sulphonamides), a strong base salt such as sodium and potassium is used and with weak basic drugs (e.g. alkaloids) a strong acid salt such as hydrochloride is prepared. The nature and type of salt formation influences the solubility, rate of dissolution, absorption, bioavailability and pharmacological response of drugs. For example, the choline and isopropanolamine salts of theophylline dissolve 3 to 4 times more rapidly than the ethylene diamine salt and show better bioavailability. The bioavailability of novobiocin from its sodium salt, calcium salt and free acid form is found to be in ratio of 50 : 20 : 1. Alkaloids are poorly soluble in water and are thus not used as such in therapeutics; instead their water soluble sulphate or hydrochloride salts are used. Various salt forms are selected to alter the pharmacological response of the drug in several ways such as to prolong duration of action (e.g. benzathine penicillin), to enhance GI stability (e.g. erythromycin estolate) and to decrease side effects. In toxicology, the type of salt form of certain metals affects the mode of action and nature of clinical signs e.g. inorganic salts of mercury primarily produce caustic and GI irritant action, where organic salts of mercury display mainly neurological signs.

3. Formulation and vehicle effects

The pharmacological response of a drug is markedly influenc by the formulation which can either increase or decrease t response by altering the chemical nature of the agent or by affecti the solubility which influences the absorption of the agent into t biological system. The effect of drug may also be influenced by t type and number of excipients (non-drug components) present the formulation. For example, physiological surfactants like b salts and lysolecithin promote absorption of hydrophobic dru such as steroids, oil soluble vitamins and griseofulvin by th micellar solubilising property. On the other hand, certain dyes (e brilliant blue) when used as colorants inhibit micellar solubilisi effect of bile acids and impair absorption of hydrophobic dru The macromolecular gums (e.g. sodium carboxymethylcellulose a methylcellulose) often used as suspending agents or viscosi imparters act as mechanical barrier to diffusion of drug by formi a viscid layer on the GI mucosa. Certain buffer systems containi potassium cations inhibit the drug absorption as seen with vitam B_2 and sulphanilamide. Some cathartic drugs, like aloes and casca show better pharmacological response than their extracted acti principles because the extractive solvents retard the absorpti and keeps the active principles in the alimentary tract until th reach the colon.

4. Physicochemical properties of drug

Various physicochemical properties of drug which affect t drug response include solubility and dissolution rate, degree ionization and particle size.

i. ***Solubility and dissolution rate:*** An important pr requisite for the absorption of a drug by all process except endocytosis is that it must be present in aqueous solution so that drug can easily come in conta with a biological membrane and rapidly permeate throu it. For a hydrophobic and poorly aqueous soluble dr (e.g. griseofulvin and spironolactone), dissolution is rate limiting step, while for a hydrophilic with hi aqueous solubility drug (e.g. cromolyn sodium a neomycin) dissolution is faster but absorption is the ra limiting step. Although solubility (oil : water partiti coefficient) of a drug is an inherent property of the dru over the years a variety of techniques have been us to enhance the solubilization and absorption of drug

ii. Degree of ionisation: Ionizable drugs can exhibit significant differences in absorption properties dependent upon the pH characteristics of the gastro-intestinal environment. Drugs containing groups un-ionised at physiological pH are usually more lipid-soluble and, therefore, are more likely to be absorbed across lipid-protein matrix of the GI- epithelium than the drugs which largely exist in ionised form at physiological pH. For example, ammonia (NH_3) is better absorbed from rumen and show rapid clinical signs in urea poisoning than ammonium ion (NH_4^+) which fail to enter the blood. Quaternary ammonium compounds remain largely ionised at plasma pH, therefore, they do not cross the blood brain barrier and have no effect on CNS. The absorption of cinnarizine largely depends on the gastric acidity of the patients. In individuals with high gastric acid content, cinnarizine shows good absorption characteristics, but in individuals showing low-gastric acid content, its absorption is reduced to a great extent.

iii. Particle size: Smaller drug particles have a higher dissolution rate and are better absorbed than the larger drug particles. The surface area of smaller particles is more so they achieve higher interaction with solvent and thus give faster dissolution. Micronized forms of several poorly aqueous soluble drugs (e.g. griseofulvin and chloramphenicol) result in a better dissolution, absorption and pharma-cological response in comparison to the simple milled form of these drugs. Micronization technique is also used to reduce the dose size of certain drugs (e.g. spironolactone) because of increased absorption efficiency.

II. Factors Related to Exposure/Administration Conditions

1. Dose

The occurrence and magnitude of a pharmacological response depends on the amount of drug absorbed into blood stream that further depends on the dose or amount administered. Normally, response of a drug increases with increase in the dose, but it occurs within a limit. A sufficiently large dose of an ordinarily harmless drug (e.g. sodium chloride) may prove fatal, but a small dose of a potent poison is without any effect and even some are used as therapeutic agents (e.g. arsenical compounds and atropine). Some drugs display dose-dependent pharmacological response and are used clinically in different dosages for different purposes. For example, low dosage of dopamine produces renal vasodilatation and natriuresis, moderate dosage produces myocardial stimulation and high dosage produces vasoconstriction. Similarly, atropine is used in lower dose as anti-secretory and spasmolytic agent and in higher dose as antidote to organophosphate poisoning.

2. Route of administration

The major routes by which a drug is administered include oral (ingestion), parental (injection), lungs (inhalation) and skin (topical). Route of drug administration primarily governs the speed and intensity of drug response. However for some drugs, the route of drug administration is the primary determinant of the type of pharmacological effect produced. For example, magnesium sulphate given orally causes purgation, applied topically on inflamed area decreases swelling, and administered intravenously produces muscle relaxation, hypotension and even cardiac arrest. Similarly, lidocaine infiltrated into the vicinity of a nerve produces local anaesthetic effect, but when given intravenously it produces antiarrhythmic effect. Ethanol applied topically has antiseptic action, but when ingested produces CNS depression and vasodilatation. Inhalation of N-acetylcysteine produces mucolysis, but when administered IV antagonizes acute paracetamol poisoning.

3. Rate of administration

Rate of drug administration governs the speed and intensity of pharmacological response. With few exceptions, rapid intravenous administration of drugs produces immediate effect and also more serious side effects than when given by slow infusion. For example, diacetylmonoxime, an acetylcholinesterase reactivator, administration by rapid IV injection produces severe side effects, but the same when given slowly is well tolerated. Urea can be highly toxic when consumed rapidly in sufficient quantity to produce high rise in rumen ammonia concentration.

4. Volume

Depending on the nature of drug and type of pharmacological response required, oral drugs are administered either in small volume or in large volume. The irritant drugs are generally administered in relatively large volume so as to avoid nausea, vomiting and gastro-enteritis. Pharmacological response of agents given by oral route also tends to be more when administered in dilute solutions because the drugs get a large surface area to undergo absorption. The opposite situation, however, occurs with agents whose pharmacological effects are only due to local irritation (e.g. emetics). Drugs given in too large volume may produce problems like excessive hydration, impaired renal clearance or vehicle toxicity.

5. Concentration

Drugs given in higher concentration by all routes creates higher concentration gradient and thus lead to faster rate of absorption and rapid onset of pharmacological effect. For some drugs, even the nature of pharmacological effect varies with concentrations used. For example, dermal application of weak solution of iodine (2.5%) produces antiseptic effect, while the strong solution of iodine (10%) produces counterirritant and parasiticide effects. Similarly, red mercuric iodide ointment at lower strength (1:40) possesses rubefacient effect, but at the higher concentration (1:4 or 1: 8) it produces vesicant and blistering effect.

6. Time of administration

Response to a drug may be related to the time of day at which it is administered. This may be related mainly to the eating and sleeping habits of the animal or to the diurnal factors. For example, in nocturnal animals such as rats, there is more food in stomach in the morning than that in the afternoon. So absorption of drug is variable depending on time of its administration. Dosing with glucocorticoids at night has been recommended for cats in order to mimic the endogenous release pattern of the hormone. In humans, hypnotics are more effective during night, because darkness itself has sedative effect and also because people sleep during night.

7. Schedule of dosage

Schedule of dosage may have an important influence on the pharmacological response of a drug. Generally for a large number of drugs, a single large dose produces more response than the same amount given in divided doses. However for a drug that is cumulative in nature, divided doses may produce more effect.

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Drugs and toxicants showing cumulative action include arsenicals, digitalis, chloroquine and copper. Warfarin, an oral anticoagulant, is less toxic in a single oral dose (Pig LD_{50} 30 mg/kg) than when it is administered in multiple doses (LD_{50} 0.05 mg/kg/day for 7 days).

8. Duration of administration

The pharmacological response of few drugs varies with the duration of drug administration. For some agents, pharmacological or toxicological effects of single administration are quite different from those produced by repeated administration. For example, single or short-term administration of propranolol produces either rise or no effect on blood pressure but on long-term administration it causes fall in blood pressure. Some organophosphorus insecticides (e.g. monocrotophos) in acute toxicity produce characteristic anti-cholinesterase manifestations like salivation, muscle tremors, dyspnoea and convulsions, but they on repeated exposure produce delayed neuropathy (e.g. paralysis of limbs). Similarly, short term exposure to elemental mercury produces corrosive and pulmonary lesions due to its irritant action, whereas long-term exposure produces mainly neurological signs.

9. Drug interactions

When pharmacological preparations are mixed indiscriminately before administration, direct drug-drug interactions may occur between the active ingredients or between the active ingredients and formulation excipients. Some drug-drug interactions may occur even within body after separate administration of agents, which may modify the response of drugs by altering either the pharmacokinetics or the pharmacodynamics of one or both drugs. Types and examples of drug interactions have already been considered in Chapter 6.

III. Factors Related to Subject

1. Species

There is wide biological diversity among different species in the rate and pattern of metabolism to detoxify a compound. In addition, differences in absorption, excretion, plasma protein binding, tissues distribution and action at receptor sites have been identified. Therefore, the type, intensity and duration of effect produced by a drug in different animal species can vary considerable. For example, belladonna or atropine is toxic to most species, but not to rabbits due to the presence of enzyme atropinase in liver and plasma which rapidly hydrolyses it. Glucuronide conjugation is less developed in cat, so cat is highly susceptible to poisoning by phenolic agents such as paracetamol. Morphine produces CNS depression in human beings, monkeys and dogs but it causes predominant CNS excitation in cats. Similarly, histamine produces vasodilatation and hypotension in dogs, cats and primates but it causes pronounced vasoconstriction and hypertension in rabbits and guinea pigs. In general, the absorption of drugs following oral administration is fast and complete in monogastric animals, and comparatively slow and incomplete in ruminants. Several drugs (e.g. antibiotics) are not effective by oral route in ruminants because they are rapidly inactivated by ruminal microflora or they may fail to diffuse into the large compartments of the GI tract.

2. Breeds

Breed differences exist in drug effect, although they have not been well described. These differences have been attributed mainly to variation in the drug disposition. For example, greyhounds are more susceptible to thiobarbiturates because their lean body weight provides little fat for the drug re-distribution. Brachycephalic breeds of dogs (e.g. Pug, Boxer, Bulldog) are more susceptible to cardiac arrhythmias (sinoatrial block) caused by acepromazine and Collie breed is more susceptible to adverse effects of ivermectin. Some breeds of pigs (e.g. Poland China and Pietrain) are found to be highly sensitive to halothane induced malignant hyperpyrexia.

3. Strains

Different strains of same animal may show variations in drug response. For example, different strains of mice vary widely in their ability to metabolise barbiturates and consequently the magnitude of pharmacological response. In humans, differences have been observed in the metabolism of drugs among different races known as ethnic variations. For example, 45% of whites in USA and Canada are slow acetylators and 55% are rapid acetylators of isoniazid. Dose adjustment in slow acetylators is essential because they are susceptible to isoniazid induced peripheral neuritis and hepatic damage.

4. Sex

Male and female animals of the same species and breed usually exhibit only slight differences in drug effect. However, sex related differences in the rate of biotransformation and hence elimination of some endogenous and foreign compounds have been identified, and are related mainly to variation in sex hormones. For example, male mice are highly susceptible to nephrotoxic effect of chloroform, but female mice show little effect. Female rats are 2 times more susceptible to red squill toxicity than male rats. In humans, ephedrine more frequently produces excitation and tremors in women than in men. In general, males are considered less susceptible to adverse effects of drugs than females because the testosterone is a known CYP3A inductor and is believed to provide some protection against adverse effects of drugs due to their faster metabolism.

5. Age

Very young (neonate) and very old (geriatric) animals are more susceptible to harmful effect of drugs when compared with adults. This is predominantly related to less efficient drug metabolising and excretory mechanisms in young and old animals. Neonates have under-developed and inefficient hepatic microsomal enzyme system, while geriatric animals have reduced liver mass, decreased hepatic blood flow and deceased microsomal enzyme activity. Similarly reduced renal functions in neonates are related to reduction in GFR and inefficient tubular carrier excretion mechanisms. The inappropriate functioning of these organs in neonates and geriatric animals tend to raise t½ of most drugs. For example half-life of chloramphenicol in piglets is about 6-7 times more than in adults. Chloramphenicol induced grey-baby syndrome in human infants is due to inadequate conjugation of the drug with glucuronic acid. Neonates and geriatrics also have low plasma albumin, as a result of which the unbound concentration of drugs which primarily binds to albumin increases (e.g. phenytoin, NSAIDs). Neonates have lesser-developed blood-brain barrier as a result many polar and water-soluble drugs (e.g. penicillins) also gain entrance into brain.

6. Pregnancy

Pregnancy causes marked hormonal and metabolic changes in body thus affecting response of certain drugs. During pregnancy, there is expansion of plasma and ECF volume;

therefore, the volume of drug distribution usually increases. Renal blood flow also increases markedly during pregnancy as a result of which the GFR and renal excretion of some drugs, particularly antibiotics, may increase. High progesterone levels during pregnancy causes increased levels of hepatic microsomal enzymes which in turn tend to enhance metabolic rate of many drugs (e.g. anticonvulsants). Examples of drugs showing of different response in pregnancy include oral anticoagulants which are more toxic to pregnant than the non-pregnant animals. The myometrial contraction produced by oxytocin is many times more in a pregnant animal than in a non-pregnant animal due up-regulation of oxytocin receptors in uterus during pregnancy. Several lipophilic drugs cross placental barrier in pregnancy and show longer duration due to slow excretion. Pregnancy has also been shown to increase the susceptibility of some pesticides in experimental animals.

7. Lactation

Similar to pregnancy, lactation alters pharmacokinetics of certain drugs and show variable response to drugs. Lactation enhances excretion of some lipophilic drugs and toxicants (e.g. DDT and polychlorinated biphenyls) in the milk. Drugs in milk may have their own unwanted effects on the suckling young ones and consumers. For example, tetracyclines administered to lactating animals produce several adverse effects such as diarrhoea, skin rashes, itching and discoloured teeth in young ones. In humans, heavy smoking by mothers has been associated with nausea, vomiting, diarrhoea and abdominal cramping in suckling babies.

8. Hormonal status

In addition to pregnancy and sex, there are a number of other hormonally dependent situations which may influence the effect of drugs. For example, hyperthyroidism and hypothyroidism can affect drug disposition and alter drug response. The elevated thyroid hormones activate some cytochrome P-450 enzyme dependent metabolic reactions (e.g. hydroxylation), while activities of some other reactions (e.g. N-demethylation) are decreased. Digoxin doses necessary to induce clinical response are in general increased in hyperthyroidism, whereas smaller doses than normal are needed in hypothyroidism. Adrenalectomised rats are generally more susceptible to adverse effects of drugs due to decreased levels of anti-stress hormone epinephrine. Stress related changes in ACTH levels also influence biotransformation of drugs.

9. Body weight and composition

In pharmacology, it is customary to adjust the dose of a drug on the basis of body weight (i.e. mg/kg), but this practice is not accurate for all situations. Differences between body weight and true lean body weight should be taken when dealing with conditions like obesity, starvation, ascites, generalised oedema and old age. In obese animals, water soluble drugs generally do not distribute into increased body fat which may result in higher than expected plasma drug concentration and hence adverse effects. In contrast, highly lipid soluble drugs (e.g. anaesthetics) are required in higher dosage in an obese animal due to their higher storage in body fat; this may increase the volume of distribution and may prolong the elimination half-life of drugs. An approximation of surface area is occasionally used for determining the dose of very toxic drugs (e.g. some anti-neoplastic drugs). It has been suggested that body surface area provides a more accurate basis for dose calculation because total body water, ECF volume and metabolic activity are better represented by body surface area.

10. Genetic status

Genetic make-up of the subject often markedly influences the response to a particular agent. This may be related to gene-related alterations in drug metabolism or alterations in tissue-receptor sensitivity. These variations may lead to genetic tolerance, intolerance or idiosyncratic reactions in susceptible individuals. Several pharmacological factors have been elucidated in man, but they are less studied in animals. For example, individuals having low level of pseudocholinesterase show prolonged muscular relaxation and apnoea produced by suxamethonium. Individuals with Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency are more susceptible to the haemolytic effect of drugs like sulphonamides, aspirin and acetanilide.

11. Nutritional status

Nutritional status of animal may markedly influence its response to a drug. A well-nourished animal easily copes with the disposition of drugs with little adverse effects. In contrast, an animal suffering from protein-caloric malnutrition often shows impairment in the absorption, distribution, metabolism and excretion processes. Starvation for few hours generally reduces blood glucose level and may result in decreased amount of glucuronides formed than the normal conditions. Protein deficiency for longer period results in reduced metabolic activity of microsomal enzyme and hence lesser availability of endogenous substrates like glutathione as a result of which drugs like paracetamol becomes more toxic. Hypoalbuminaemia increases portion of free and unbound drugs as a result of which agents like warfarin show enhanced pharmacological response. Deficiency of vitamins, especially antioxidant vitamins E and C, can result in increased damage from free radicals. Dietary deficiency of vitamins (e.g. vitamins A, B_2, B_3, C and E) and minerals (e.g. Fe, Ca, Mg, Cu, and Zn) retard metabolic activity of several enzymes.

12. Emotional status and temperament

Efficacy of a drug may be affected by the emotional status and temperament of the animal. This is particularly applicable to the centrally acting drugs. For example, an excited animal may require higher dose of a CNS depressant than the animal which is already depressed. Toxicity of drugs like amfetamine and other CNS stimulants is affected by crowding (more number of animals per cage), size of cage, bedding and handling of animals. Stress and anxiety generally promote gastric emptying, while depression retards it. Anything that emotionally disturbs the animal may influence its physiological reactions and, thus, possible changes in reaction to foreign chemicals.

13. Tolerance

Acquired tolerance may develop after repeated or prior exposure to a given drug or to a closely allied drug that resembles the former in its pharmacological activity. Tolerance has been related to the changes either in the disposition of drug that reduces effective drug concentration in the system (called pharmacokinetic tolerance) or to the target system that renders the system least responsive (called pharmacodynamic tolerance). Tolerance is frequently seen with repeated uses of drugs such as opioid analgesics, benzodiazepines and other psychotropic drugs. Barbiturates increase their own metabolism by causing microsomal enzyme induction (called auto-induction) thus producing tolerance.

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14. Individual variations

Individual variations (Idiosyncrasy) to drugs are common in a homogenous population. Within a population, some animals are hypersensitive and some are hyposensitive to drugs. These individual variations are unrelated to the pharmacological profile and are not attributable to genetic or other biological factors. For example, first generation antihistamines produce variable degree of drowsiness and sedation in individuals in a population.

15. Disease and pathological states

Pathological conditions, particularly those affecting major organ systems or altering fluid pH and electrolytes can substantially alter both the pharmacokinetic fate and the pharmacological action of many drugs.

i. Liver diseases: Liver diseases may reduce activity of microsomal enzymes, thus altering biotransformation and action of drugs and toxicants. Liver diseases can also reduce synthesis of protective binding molecules (e.g. glutathione) allowing increased adverse effect of drugs. Reduce synthesis of plasma proteins, particularly albumin, may alter the drug binding capacity. For example, hepatitis impairs biotransformation and prolongs action of injectable anaesthetics as a result of which ultra-short acting barbiturates become toxic. Impaired biotransformation also leads to enhanced activity of several drugs like diazepam, tolbutamide, morphine, digitoxin and phenobarbital. In contrast, pro-drugs needing hepatic metabolism for activation (e.g. prednisone, sulindac) are less effective in hepatitis and should be avoided in such patients.

ii. Kidney diseases: Kidney diseases often result in decreased clearance and thus slower removal of drug from the body. A normal dosage regimen in such cases often leads to accumulation of drug in body and ultimately to toxicity. Drug induced toxicity in renal insufficiency may also result from either derangement of acid-base balance or increased sensitivity to the drug due to uraemia-induced alterations in tissue receptors. For example, in renal patients with impaired function some antibiotics like streptomycin, gentamicin and penicillins accumulate to toxic levels in body. Urinary antiseptics like nalidixic acid, nitrofurantoin and methenamine do not achieve high levels in urine in renal insufficiency and are likely to produce systemic toxicity.

iii. Other diseases: Several other pathological states may influence the pharmacological response of a number of drugs. Conditions causing diarrhoea may decrease absorption of drugs by enhancing gut motility, while those causing constipation may enhance absorption of drugs. Cardiovascular insufficiency due to either cardiac failure or circulatory shock potentially affects disposition of drugs by altering drug distribution and elimination. Decompensated heart usually results in decreased volume of distribution and decreased clearance of several drugs like lidoocaine, procainamide and quinidine. Neurological disturbances may influence animal's response to several CNS acting drugs like morphine. In idiopathic canine epilepsy and epileptiform conditions, phenothiazine neuroleptics often precipitate seizures. Inflammation of meninges may precipitate toxicity of several drugs (e.g penicillins) by allowing their penetration into the brain Thiazides induce more diuresis in oedematous patients

IV. Factors Related to Environmental Conditions

1. Temperature

Environmental temperature has been demonstrated to influence pharmacological or toxicological response of some drugs by influencing their absorption, metabolism and excretion. Such changes are apparently related to the temperature dependent biochemical reactions responsible for the effect and disposition of the chemical. For example, low ambient temperature usually enhances metabolism of drugs because the metabolic activity increases in cold conditions to keep the animal's body warm. The high ambient temperature favours administration of inhalant volatile anaesthetics by open drop method mainly by increasing their volatility and vapour pressure. On the other hand, low ambient temperature can increase duration of action and toxicity of general anaesthetics because they impair the thermoregulatory mechanism and lower the body temperature. Ethanol toxicity also increases in winter because cutaneous vasodilatation caused by ethanol results in excessive heat loss on exposure to cold. Toxicity of some insecticides belonging to organochlorine and pyrethroid groups shows negative correlation with environmental temperature (more toxic in winter). However, pesticides those cause oxidative uncoupling action (e.g. dinitrophenols) produce hyperpyrexia and are more toxic in hot conditions.

2. Atmospheric pressure and altitude

Atmospheric pressure and altitude can affect the pharmacokinetic profile of some drugs. The changes in response to drugs and poisons are generally associated with changes in the environmental oxygen tension. High altitude with low atmospheric pressure reduces the potential of body to oxidise drugs, as less amount of oxygen is available to the body. Low oxygen concentration resulting from high altitude is also an important factor in the toxicity of compounds which primarily affect cardiovascular functions. For example, carbon monoxide, barbiturates and cyanide are more toxic in low oxygen conditions. Greater toxicities of digitalis, red squill and strychnine are observed at high altitude as compared to low altitudes.

3. Light and other radiations

Light related factors affect biological rhythms in some animals and influence susceptibility to drugs. In the rat and mouse, the activities of cytochrome P-450 are greatest at the beginning of the dark phase. Radiation exposure is known to affect blood-tissue barriers, modify enzyme systems and produce disturbances in the normal excretory pattern of numerous species. Certain drugs (e.g. phenothiazine, demeclocycline and tar products) show photosensitization reactions when the individual is exposed to sunlight or UV radiations. Toxicity of atropine usually increases if the intoxicated animal is exposed to direct sunlight because of its mydriatic and cycloplegic effects on eyes. Whole body irradiation increases the toxicity of CNS stimulants but decreases that of CNS depressants.

4. Environmental pollution

Several chemicals in environment, especially those in the air and water, can substantially alter the pharmacokinetics and

pharmacodynamics of many drugs. Chemicals may influence the response in a variety of ways by affecting biotransformation (induction or inhibition of hepatic microsomal enzymes), absorption, distribution, excretion and affinity for receptors. For example, exposure to tobacco smoke is well known to enhance drug metabolism through induction of microsomal enzymes. Chronic exposure to air pollutants (e.g. sulphur oxide gases) causes thickening of respiratory tract mucous membranes which may impair absorption and action of some inhalant drugs. Inhalation of dusts containing minerals and organic matter for long periods may produce pulmonary diseases in humans and animals.

5. Relative humidity

Relative humidity might influence the reaction of an animal to a drug, especially those applied by dermal route. For example, dermal absorption of some ectoparasiticides (e.g. organophosphorus insecticides) increases in hot and humid environment because more blood is diverted to skin (so rapid absorption) to affect cooling.

6. Ventilation

Poor air circulation in sheds and barns may alter response of some agents. For example, silo gases accumulate in confined spaces and settle to low spots to cause toxicity in animals. Poorly ventilated shed often pre-disposes to toxicosis and diseases from accumulation of poisonous gases such as carbon monoxide or natural gases which contain methane.

7. Management and social factors

Animal husbandry practices and social factors can modify the pharmacological or toxicological response of some chemicals. These include improper handling of animals, inadequate housing (overcrowding), inappropriate size of cages, material of mangers and cages, frequent visit of strangers, type of bedding material, use of disinfectants, etc. For example, toxicity of drugs like amfetamine and other CNS stimulants is enhanced by crowding (more number of animals per cage) and excessive handling of animals. External disturbances like sound and visitors generally aggravate poisoning of convulsants like strychnine.

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CHAPTER 8 ADVERSE DRUG EFFECTS AND TREATMENT OF POISONING

Adverse drug effects
- Types of adverse drug effect
  - Type A or Pharmacological adverse drug effects
  - Type B or Non-pharmacological adverse drug effects
  - Type C or Chronic effects
  - Type D or Delayed effects
  - Type E or End-of treatment effects
- Prevention of adverse drug effects

Treatment of poisoning
- Prevention of further absorption of poisons
  - Non-specific treatment
  - Specific treatment
- Alteration of distribution and action of poisons
  - Alteration of distribution of poisons
    - Non-specific treatment
    - Specific treatment
  - Alteration of action of poisons
    - Non-specific treatment
    - Specific treatment
  - Hastening elimination of poisons
    - Non-specific treatment
    - Specific treatment

Management of poisoning

ADVERSE DRUG EFFECTS

Adverse drug effect (ADE) is an unwanted or unintended effect of a drug that is harmful to the patient. In clinical practice, the adverse drug effect may occur even at therapeutic doses and vary in severity from mere inconvenience to serious organ dysfunction or even death. In veterinary medicine, ADE occurs mainly due to administration of an inappropriate drug in an inappropriate dosage to the inappropriate species. Use of human-label drugs in animal practice for which drug safety and efficacy data have not been established, use of multiple drugs simultaneously, or fixed dose drug combinations in animals also lead to adverse drug effects. As more and more drugs are marketed and prescribed every year, the occurrence of adverse drug effects is continuously increasing in both human and veterinary practices. In clinical practice a distinction should be made between tolerable adverse effects and serious adverse effects (SAEs). Generally, any event which causes death, disability, permanent impairment or damage, birth defects, or requires hospitalization is considered an SAE. Depending on the condition, the drug induced adverse effects may require corrective treatment, adjustment in drug dosage, cessation of therapy or use of an alternative drug. These also require better approaches for the reporting, assessment and management of drug induced diseases and guidelines for the future use of the drug.

Adverse drug reactions: Adverse drug reaction may be defined as a noxious change that occurs at normal doses and excludes trivial and expected side effects and ill effects of over dosage. However, the term is often used interchangeably with the adverse drug effect.

Types of Adverse Drug Effect

Adverse drug effects were earlier classified in 1970s into two types i.e. Type A and Type B; however this classification was proved insufficient as it failed to include all types of drug reaction. Later on Rawlin and Thompson devised another classification scheme in 1991, which continues to be the most frequently used and is presented below with some modifications. Still there are many adverse reactions, which cannot be classified because the mechanisms responsible for them are not known. These reactions are very rare and not reproducible in animal models.

I. Type A or Pharmacological adverse drug effects
1. Exaggerated desirable effect
2. Side effects
3. Secondary effects
4. Drug interactions

II. Type B or Non-pharmacological adverse effects
1. Hypersensitivity
2. Idiosyncrasy
3. Photosensitization
4. Intolerance

III. Type C or Chronic effects
1. Drug dependence

IV. Type D or Delayed effects
1. Carcinogenicity
2. Mutagenicity
3. Teratogenicity

V. Type E or End-of treatment effects
1. Iatrogenic diseases

I. Type A or Pharmacological Adverse Drug Effects

Type A or Pharmacological adverse drug effects are based on the known properties of the drug and are generally a result of the augmented pharmacological effects of the drug. These

adverse effects are mostly associated with prescription errors and are more closely related to the dosage of drug. These effects are well described in the literature including textbooks, handbooks and physician's desk reference and can be prevented or minimized by taking certain precautions. Type A adverse effects occur more commonly (about 80%) than any other type of ADE, but they are readily reversible on reducing dose or withdrawing treatment and the incidence of mortality in such reactions is low. Pharmaceutical companies strive to work out the Type A adverse effect profile of a drug before it is marketed, but the complete range of adverse effects cannot be found before licensing and an efficient post-marketing surveillance is needed.

1. Exaggerated desirable effects

Many adverse effects are simple extensions of the desirable or primary pharmacological effects of drugs. These effects are mostly observed with over dosage or after prolonged use of a drug. This form of ADE can be anticipated and avoided by reduction of the dose. For example, haemorrhage occurs on over dosage of warfarin, and water and electrolyte depletion can occur on over dosage of diuretics. Bradycardia with beta-blockers is another example of an exaggerated response to a drug's primary activity.

2. Side effects

Side effects are unwanted but unavoidable pharmacological effects of drugs which occur at normal therapeutic doses. Similar to desirable pharmacological effects, many ADEs are extensions of side effects and are mostly observed with over dosage or prolonged use of drugs. They can be predicted from the pharmacological profile of a drug and often minimised by adjusting the dosage. Examples of ADEs associated with side effects of drugs include excessive sedation with some antihistamines, constipation with anticholinergics, and postural hypotension with antihypertensives. There is a significant individual variation in side-effects of any drug, and between different drugs within the same therapeutic class for an individual patient. A patient may therefore be able to tolerate sedation induced by antihistaminics better than another patient.

3. Secondary effects

Secondary effects are the indirect consequences of a primary action of drug. These are also predictable, but usually cannot be controlled by dosage adjustment. However, substituting or adding another drug to the therapy may reduce these effects. For example, broad-spectrum antimicrobial drugs cause diarrhoea due to suppression of gut microflora and aspirin and related drugs induce gastrointestinal bleeding and ulceration. Clinical use of an immunosuppressant frequently enhances susceptibility to infection due to depression of defence mechanisms.

4. Drug interactions

A drug interaction is a condition in which a drug (or food substance) affects the activity of another drug i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. Drug interaction is a very common mechanism of drug induced adverse effects and is often a result of wrong prescription. Most drug interactions are well known and can be predicted from the pharmacology of drugs. Adverse drug interactions can be easily avoided by following guidelines for the safe use of drugs. Examples of adverse drug interactions include theophylline toxicity in the presence of erythromycin therapy and warfarin toxicity in presence of aspirin therapy. MAO-Inhibitors (e.g. selegiline) produce life threatening hypertension in presence of cheese in food (called cheese reaction) and metronidazole produces disulfiram-like reaction in presence of alcohol.

Table 8.1: Comparative features of Type A and Type B of adverse drug effects (ADEs).

Features	Type A ADE	Type B ADE
Predictable from pharmacology	Yes	No
Dose dependent	Yes	No
Incidence	High	Low
Morbidity	High	Low
Mortality	Low	High
Management	Dose adjustment	Treatment cessation

II. Type B or Non-Pharmacological Adverse Drug Effects

Type B or Non-pharmacological adverse drug effects (adverse drug reactions; ADEs; ADRs) occur as result of an abnormal sensitivity or reactivity of an individual to a chemical substance and have little/no relationship with the pharmacological effect(s) of drug. These adverse effects are not predictable on basis of the pharmacological profile of the drug and, therefore, cannot be anticipated unless the animal has a history of being sensitive to some drugs. Type B reactions have been attributed to genetic or idiosyncratic factors and hypersensitivities. Some species differences reflecting different receptor sensitivity may also be considered in type B reactions. Non-pharmacological drug effects are more commonly observed in human medical practice and do not appear to have a high incidence in veterinary medicine. Nevertheless, non-pharmacological ADEs can occur in animals and can have serious and even fatal consequences.

1. Hypersensitivity/Allergic Reactions:

Hypersensitivity or allergic drug reactions are immunologically mediated untoward drug effects which are unrelated to the pharmacodynamic profile of drugs. These effects are usually not dose related as severe manifestations can occur even after exposure to very limited amounts of antigenic drugs. Most of drugs are not immunogenic by themselves but can act as incomplete antigens or haptens, which become complete antigens in combination with a body protein. Therefore in drug allergy, as in other types of allergic manifestations, the first exposure leads to sensitisation to the drug and subsequent exposure results in allergic reactions (a latent period of at least 1 to 2 weeks is required after the first exposure). Chemically related drugs (e.g. penicillins) often show cross-sensitivity.

The chief target organs of drug allergy are skin, respiratory tract, GI tract, blood and blood vessels. The overall incidence of drug-allergy in veterinary species is low (about < 10% of all drug-related adverse effects). The great majority of reactions are relatively harmless skin manifestations; serious life threatening reactions are rare. Allergic drug reactions are reversible; most mild reactions subside by themselves on discontinuation of therapy and do not require specific treatment.

Allergic reactions are further classified into 4 types: Types I, II, III and IV. First three reactions are humoral (antibody) mediated, while the fourth reaction is cell mediated.

a. Type I/Anaphylactic reaction: Type I or Anaphylactic (immediate) hypersensitivity reaction is an acute multisystem severe life threatening reaction that occurs within minutes of a drug exposure (the onset may be delayed for ≥ 1 hour after administration of relatively insoluble, repository dosage form such as benzathine penicillin). It involves formation of specific antibodies called 'reagins' which belong to the IgE class. The reaginic antibodies get fixed to the mast cells and basophils and on re-exposure to the drug get attached to it. The antigen-antibody (AG: AB) reaction on the cell surface, particularly when antigen cross-links two IgE molecules, releases mediators like histamine, serotonin, leukotrienes, prostaglandins, platelet activating factors, cytokines and some enzymes (Figure 8.1). These mediators then react with target organs (blood vessels, GI tract, respiratory tract, skin, etc.) to produce acute inflammation and systemic effects. The manifestations associated with mediator release can be instantaneous (e.g. anaphylaxis), delayed for 2 to 4 days, or biphasic with both immediate and delayed reactions (recurrence of symptoms within 72 hours with no further exposure to the allergen).

Important manifestations of anaphylaxis seen after administration of drug may include hypotension, bronchospasm, angioedema, urticaria, erythema, pruritus, pharyngeal and/or laryngeal oedema, dyspnoea, cyanosis, cardiac arrhythmias, vomiting, colic and hypersensitivity. Death in severe anaphylaxis is attributable to cardiac arrest, shock or asphyxia. Anaphylaxis most commonly occurs following parenteral administration of drugs but may also occur after their inhalation or oral exposure. Drugs inducing type I hypersensitivity reactions may include β-lactam antibiotics, and certain carriers and solubilisers. Cross-reactivity between closely related compounds (e.g. β-lactam antibiotics such as penicillins and cephalosporins and any of chemically related sulphonamides) might result in similar response. Many foods (e.g. peanut, shellfish, fish, milk and egg) can also trigger anaphylaxis.

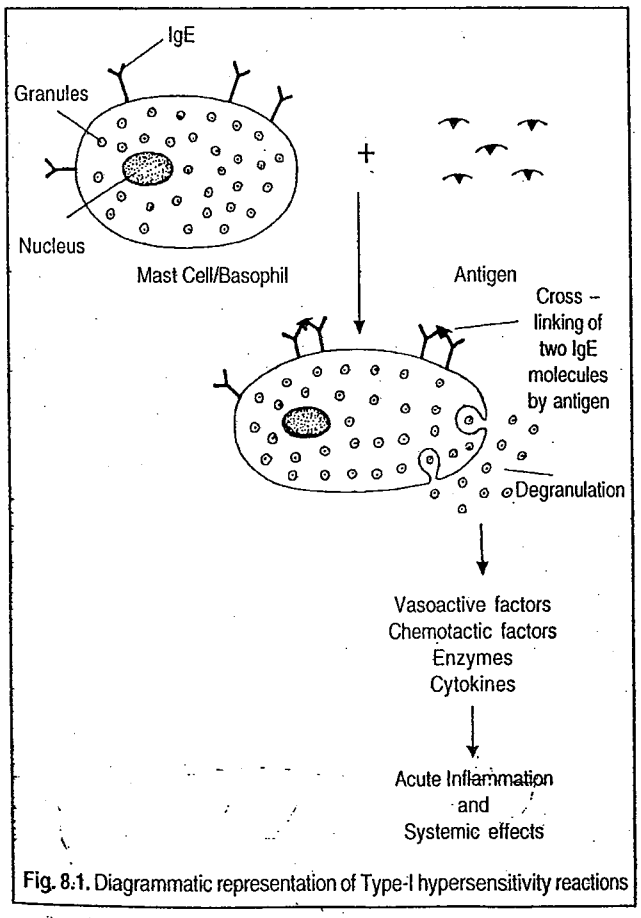


Fig. 8.1. Diagrammatic representation of Type-I hypersensitivity reactions

Pathophysiologically, a non-immune (non-IgE) mediated mast cell degranulation can also occur by certain drugs resulting in a reaction called anaphylactoid reaction or pseudo-anaphylaxis. The anaphylactoid reaction mimics anaphylaxis in the symptoms, treatment and risk of death, but it occurs as a result of direct mast cell degranulation by the drugs. Unlike the anaphylaxis, pre-exposure to drug in anaphylactoid is not necessary, but it is partly dose-dependent and predictable. Cationic drugs such as radiocontrast dyes and amphotericin B are capable of causing direct mast cell degranulation and thus an anaphylactoid reaction in animals. Life saving treatment of anaphylaxis and anaphylactoid reaction includes systemic administration of epinephrine or other β-adrenoceptor agonists. Antihistamine drugs are also beneficial in some of these reactions. If the reaction is not fatal, the manifestations subside over hours. A small test dose can be administered in suspected cases to determine the safety of drug.

b. Type II/Cytolytic reactions: Type II or Cytolytic reactions (Delayed IgG/IgM mediated reactions, autoallergy) occurs when a drug combines with a protein of specific tissue cell, especially that in the blood, and the drug-protein combination acts as an antigen. The body no longer recognises the protein as self and treats it as a foreign protein and forms antibodies (IgG or IgM) against it. The antibodies bind to the target cell and on re-exposure to the drug reacts with it. The AG: AB combination leads to activation of complement system that damages cells (cytolysis). The major target tissues for cytolytic reactions are cells in the circulatory system.

Disorders associated with Type II reactions include autoimmune haemolytic anaemia, thrombocytopenia, erythroblastosis fetalis, acute rheumatic fever, Graves disease (goitre) and myasthenia gravis. Organ damage (e.g. liver, kidney and muscles) and collagen diseases (e.g. systemic lupus erythematous) may also occur due to Type II reaction. Drug like quinine, penicillin and phenacetin adsorb onto the surface of red cells and cause haemolytic anaemia. Sulphonamides, phenylbutazone, phenothiazine and possibly chloramphenicol may cause agranulocytosis by binding to the granulocytes, and may provoke thrombocytopenia. Drugs like cephalosporins are reported to modify red cell membranes in such a way that the cells passively adsorb antibodies and phagocytic cells then remove

Table 8.2: Anaphylaxis in domestic animals.

Species	Primary target organ(s)	Important clinical signs	Pathology	Major mediators
Horses	Respiratory tract, intestine	Cough, dyspnoea, diarrhoea	Emphysema, intestinal haemorrhage	Histamine, bradykinin, serotonin.
Ruminants	Respiratory tract	Cough, dyspnoea, Collapse	Emphysema, lung oedema, haemorrhage	Histamine, leukotrienes Bradykinin.
Dogs	Liver, hepatic vein, respiratory tract	Collapse, vomiting, diarrhoea	Hepatic engorgement, visceral haemorrhage	Histamine, leukotrienes.
Cats	Respiratory tract, intestine	Dyspnoea, vomiting, diarrhoea, pruritus	Lung oedema, intestinal oedema	Histamine, leukotrienes.
Swine	Respiratory tract, intestine	Cyanosis, pruritus.	Systemic hypotension	Histamine.

them. Auto-immune reactions to drugs usually subside within several months after the offending drug is withdrawn. Immunosuppressive therapy is required only when the autoimmune response is unusually severe.

c. ***Type III/Immune-complex mediated reactions:*** Type III hypersensitivity reactions (Immune-complex mediated reactions) occur when the antigen (drug or foreign protein) and the antibody (mainly IgG) form large immune complexes and activate the complement system. The complement-activating immune complexes are deposited in tissues giving rise to generation of certain chemotactic peptides, which attract neutrophils. The neutrophils release free radicals and enzymes into tissues and so cause inflammation and tissue destruction. Local reactions occur when immune complexes are deposited within tissues. Systemic reactions occur when large quantities of immune complexes are formed and deposited in the walls of blood vessels causing damage to small blood vessels. Severe skin reactions may follow deposition of antibody-drug complexes in blood vessels of the dermis. Circulating immune complexes are also deposited within glomeruli in the kidney leading to glomerulonephritis. If the complexes bind to blood cells anaemia, leucopenia or thrombocytopenia result.

Disorders associated with Type III reactions include serum sickness, Arthus reaction and systemic lupus erythematosus. Serum sickness (the name is derived from the fact that allergic response results from damage to blood vessels by immune complexes) is a systemic reaction that occurs primarily as a result of exposure to antibodies derived from animals (e.g. antiserum). Certain drugs (e.g. sulphonamides, penicillins, certain anticonvulsants and iodides) are also capable of producing serum sickness. The disorder is characterized by signs and symptoms commonly associated with allergic reactions or infections such as rashes, itching, arthralgia, fever and swollen lymph nodes (lymphadenopathy). Other signs include hypotension or even shock and an enlarged spleen, glomerulonephritis and proteinuria. The serum sickness is normally produced after 1-3 weeks of initiation of drug therapy; however, an accelerated form of the reaction (onset 2-3 days) may occur in animals which have been previously sensitized to the drug. While serum sickness may mimic an allergic reaction, it is different from true anaphylaxis, since the symptoms are not instantaneous. Immediate withdrawal of the offending drug is necessary to reduce severity of the serum sickness. The symptoms usually subside in 1-2 weeks on their own, but glucocorticoids may be prescribed in severe forms. Use of antihistamines may also provide relief from the reactions.

d. ***Type IV/Delayed type hypersensitivity reactions:*** The Type IV or delayed type hypersensitivity (tuberculin type, antibody independent) reactions develop several hours (2-3 days) after exposure to the drug (antigen) and are mediated through production of sensitised T-lymphocytes (and macrophages) carrying receptors for the antigen. On contact with the antigen these T cells produce cytokines, which attract granulocytes and generate an inflammatory response. The reaction generally takes >12 hours to develop. Disorders associated with Type IV reactions include contact dermatitis, multiple sclerosis, some peripheral neuropathies and diabetes mellitus type I. Contact dermatitis results from the exposure to allergens (allergic contact dermatitis) or irritants (irritant contact dermatitis) with manifestations of lesions varying from mild erythema to severe erythematous vesiculation. If the exposure to the allergen persists hyperkeratosis, acanthosis, and dermal fibrosis may eventually result. Sources of contact allergen in animals may include insecticides (e.g. sprays, dips and flea collars), dermatological drugs (e.g. creams and ointments), plants (e.g. poison ivy and poison oak) and paints. Treatment of Type IV reactions involves withdrawal of the offending drug or irritant and use of corticosteroids in severe cases.

2. Idiosyncrasy

Idiosyncrasy (Greek: *idiosynkrasia* = a peculiar temperament) is a qualitatively abnormal reactivity of an individual to a chemical that has no obvious relationship to either dose or duration of therapy. Similar to other Type II reactions, it is unrelated to the pharmacological profile of the drug and occurs only in a small population of individuals. Much of the prevalent thinking about mechanisms of drug idiosyncrasy has been based on hypotheses that the reactions have a metabolic basis involving drug metabolism polymorphisms or from a specific immune response to the drug or its metabolite(s). However only for very few drugs,

the convincing evidence exists for either of these mechanisms. Several studies in animals suggest the possibility that some events during the course of therapy render tissues peculiarly susceptible to adverse effects of the drug leading to the idiosyncratic reaction. Idiosyncratic reactions are harmful, sometimes fatal, and may appear even in low doses. The type and severity of the reactions vary with the drug and the affected individual, but only some of these result in permanent disability or death. Idiosyncratic reactions occur more frequently with exposure to new drugs, as they have not been fully tested and their full range of possible side effects has not been discovered. Many drugs precipitate idiosyncratic reactions including some antipsychotic drugs such as chlorpromazine and clozapine which cause infrequent reactions such as rhabdomyolysis (breakdown of striated muscle) and liver toxicity in humans. Halothane produces malignant hyperthermia and hepatitis, and sulphonamides produce haemolytic anaemia in some individuals.

3. Photosensitization

Photosensitization (Photosensitivity) is an adverse effect in which lightly pigmented skin becomes hypersensitive to sunlight. Photosensitization in animals occurs due to presence of a photodynamic agent (drug or it metabolite) in the peripheral circulation that absorbs light and initiates some chemical reactions in the skin. Tissue injury probably results from production of reactive oxygen intermediates and/or alterations in cell membrane permeability. Photosen-sitization occurs worldwide and can affect all species, but is most commonly seen in sheep, cattle, goats and horses. Clinical manifestations of photosensitization include erythema, oedema, blistering, serum exudation, scab formation and skin necrosis in lightly pigmented areas such as ears, eyelids, muzzle, etc. Drugs causing photosensitization in animals include phenothiazines, sulphonamides and tar products. Several plants including species of *Trifolium* (clovers), *Medicago* (alfalfa), *Polygonum, Brassica, Heliotropium* and *Lantana* have been incriminated as photosensitizers. Treatment of photosensitization involves confinement of the affected animals to shaded places and parenteral administration of corticosteroids in severe cases.

Occasionally some drugs or their metabolites induce photoallergy by stimulating a cell-mediated immune response. It probably is due to photosensitising drug forming an antigen by absorption of sunlight and combination with skin protein. This results in formation of papular or eczematous contact dermatitis like condition. It occurs mostly in human beings and may be seen with drugs like sulphonamides, sulphonylureas and chloroquine.

4. Intolerance

Intolerance is the appearance of an unexpected and exaggerated pharmacological response (desirable or undesirable) in an individual at therapeutic doses. It is converse of tolerance and means low threshold to the pharmacological actions of a drug. Drug intolerance is uncommon and idiopathic, thus extremely difficult to predict except in individuals with a prior history or a family history of intolerance to that specific drug. The drug intolerant individuals fall on the extreme left side of the Gaussian frequency distribution curve for sensitivity to the drug. Adverse drug effects in hypersensitive individuals can be avoided or minimised by choosing some alternating drug or minimizing drug dosage. The exact cause of intolerance is not clear, but some drug intolerances are known to result from genetic variants of drug metabolism. For example, intolerance to analgesics, particularly NSAIDs, in humans and animals mainly occur due to variations in the metabolism of arachidonic acid. Some animals show hypersensitivity to ectoparasiticides (e.g. organophosphates and pyrethroids) and exhibit toxic signs even at the recommended concentrations.

III. Type C or Chronic effects

Type C adverse effects are associated with long-term drug therapy. They are well known and can be anticipated from the pharmacology of the drug. The Type C adverse drug effects primarily include drug dependence.

Drug Dependence

Drug dependence may be defined as a state produced by repeated administration of a drug in which the use of a drug becomes necessary for either psychological or physical well being of an individual. Drug dependence is mainly seen in human beings who continuously use mood or behaviour altering drugs (e.g. ethanol and narcotics) for non-medical or recreational purposes. It may also be seen with certain therapeutic agents (e.g. analgesics, antihypertensives and hypnotics), which are continually taken for longer periods. In veterinary medicine, drug dependence has limited importance because only a few drugs are prescribed for a duration that produces dependence. Drug dependence has been found to be of two distinct types as recognised in human beings: psychological and physical.

i. ***Psychological dependence:*** It is a state of mental dependence where the individual believes that his well-being is achieved only through the action of a drug, although the drug is not essentially required by the body. The intensity of psychological dependence may vary from desire (habituation) to craving (addiction). If the patient suddenly stops the drug, it can lead to psychological withdrawal symptoms (e.g. anxiety, anorexia, insomnia and depression), but no abstinence syndrome. The psychological dependence is believed to be strongly associated with the dopaminergic system of the brain's reward system as seen with drugs like cocaine and amfetamine. Other drugs producing mainly psychological dependence include cannabis, marijuana, phencylidine, lysergic acid diethylamide (LSD), mescaline and psilocybin. It is suggested that psychological dependence is not limited only to substances; even activities and behavioural patterns such as eating, reading, gambling, etc. can cause a mental dependence.

ii. ***Physical dependence:*** It is an altered physiological state where the individual is dependent upon the drug for the normal physiological functioning. In contrast to the psychological dependence, an abrupt discontinuation or dosage reduction of the drug results in a characteristic withdrawal (abstinence) syndrome, which constitute the only evidence for physical dependence. Abstinence syndromes are drug- or drug class-specific and may vary considerably based on the amount and frequency of drug use and on patient characteristics. Physical dependence can develop from the repeated therapeutic

use of certain drugs as well as misuse of recreational and addictive drugs. Examples of drugs producing physical dependence include nicotine, opioids, barbiturates, benzodiazepines, steroids and ethanol. Physical dependence is usually managed by a slow dose reduction of the drug over a period of weeks, months or sometimes longer and often administration of another drug, which is less harmful. Response of treatment largely depends on the drug, dose and individual.

Habituation and Addiction: Drug habituation (habit) is a condition resulting from the repeated consumption of a drug, where there is a desire but no compulsion to continue taking the drug or to increase its dose. There is also no dependence and no abstinence syndrome in case of habituation. In contrast to habituation, the drug addiction is a pathological or socio-behavioural abnormal condition where there is a strong craving and compulsion to continue taking the drug, despite its negative or dangerous effects on the body. Both psychological dependence (essentially) and physical dependence (generally) constitute important components of drug addiction, but dependence does not essentially lead to addiction. For example, the therapeutic uses of certain steroids, antidepressants, antihypertensives and even antihistamines all produce physical dependence with characteristic withdrawal syndromes, but there is no strong motivation to continue the use of these drugs. Some drugs and substances such as coffee produce psychological dependence, but there is no addiction. In a similar way, addiction can also occur without appreciable physical dependence (e.g. cocaine and phencyclidine), but it is not clear whether addiction ever occurs without psychological dependence. In literature, the terms drug dependence and drug addiction are often used interchangeably, but they carry different meanings and must be considered separately. The terms habituation and addiction are less used in pharmacology to describe action of drugs.

IV. Type D or Delayed effects

Type D effects refer to carcinogenic, mutagenic and teratogenic effects. These effects are delayed in onset and are rare since extensive studies are done before a drug is licensed. However, the delayed effects are very important adverse drug effects and must be identified before a drug is recommended for therapeutic use.

1. Carcinogenicity

Carcinogenicity refers to the capacity of a drug to induce cancer. Exposure to certain drugs, chemicals and physical agents, called carcinogens, are associated with high incidence of some types of cancer. Carcinogens are classified according to their mode of action as genotoxic or non-genotoxic carcinogens. Genotoxic carcinogens initiate carcinogenesis by direct interaction with DNA, resulting in DNA damage or chromosomal aberrations, whereas non-genotoxic carcinogens do not directly affect DNA but act in other ways to promote growth. Examples of genotoxic carcinogens include chemical agents like N-nitroso-N-methylurea (NMU) or non-chemical agents such as ultraviolet light and ionizing radiation and those of non-genotoxic carcinogens include hormones and some organic compounds. Drugs recognised as being carcinogenic are alkylating agents (e.g. cyclophosphamide), androgens, oestrogens, nitrofurazone and metronidazole. Development of cancer induced by drugs usually takes very long time (10-14 years). Carcinogenicity is considered an important and serious adverse effect in human beings but has limited importance in veterinary species because of shorter life span of animals.

2. Mutagenicity

Mutagenicity refers to the capacity of a drug to cause abnormalities of genetic material (genes, chromosomes) of cells so that a permanent change in the hereditary constitution (mutation) occurs. Usually oxidation of drugs results in production of reactive intermediates, which affect the genes and may cause structural changes in the chromosomes. If mutation occurs in reproductive cells (ova or spermatozoa), a hereditary defect appears in next generation(s). In case of non-reproductive cells, mutation may lead to other adverse effects like carcinogenesis. As many mutations cause cancer, mutagens are typically also carcinogens. Similar to carcinogenesis, drug-induced mutagenesis has limited importance in veterinary species. Drugs known to be mutagenic include alkylating agents (ethylnitrosourea), vinca alkaloids, 5-bromouracil, metronidazole and acridine dyes. Ionizing radiations (X-rays, gamma rays and alpha particles) and ultraviolet rays also induce mutation. Mutagenic drugs are not to be recommended in pregnant animals.

3. Teratogenicity

Teratogenicity (Dysmorphogenicity) refers to the capacity of a drug to cause foetal malformation when administered to pregnant animals. Teratological changes in foetus may occur as a result of physiological and biochemical alterations in the formation of cells, tissues and organs, which are manifested externally as abnormal development of organs or systems. The type of malformation depends on the drug as well as the stage of exposure to the teratogen. Although embryonic development may be affected at any time during the whole gestation period, exposure to drugs during the organogenesis phase of gestation is considered most vulnerable period for foetal malformation. Therefore, use of drugs should be avoided during the first trimester of pregnancy, unless very necessary. A wide range of different chemicals and environmental factors are suspected or are known to be teratogenic in humans and in animals. These include alkylating agents, androgens, progestins, antithyroid drugs, diethylstilbestrol, captopril, coumarin, corticosteroids, isotretinoin, hexachlorophene, lithium, methimazole, nitrazepam, tetracyclines, thalidomide, valproic acid and warfarin.

V. Type E or End-of treatment effects

1. Iatrogenic Diseases

Iatrogenic (Physician induced) diseases are drug–induced diseases, which occur occasionally after the therapy is terminated or sometimes even during course of therapy. Such diseases generally occur due to alterations in the concentration of some endogenous substances (e.g. hormones and neurotransmitters) and body functions and may persist even after the offending drug has been withdrawn or largely eliminated from the body. Iatrogenic diseases may also include various nosocomial diseases (acquired in hospital) and those induced by the medical negligence, medical error or misjudgement, and contravention of contra-indications. Knowledge about disease process, pathophysiology of patient and pharmacology of drug enable

practitioners to avoid or minimise the drug-induced diseases. Examples of iatrogenic diseases include parkinsonian syndrome produced by prolong use of phenothiazines, hypoadrenocorticism induced by glucocorticoids and gastric ulcers caused by NSAIDs. In a similar way, fluoroquinolones produce cartilage damage and joint destruction in pups and foals, sulphasalazine produce dry eyes in dogs, cortisone produce laminitis in horses and ponies, and chloramphenicol produce myelosuppression and aplastic anaemia in human beings.

Prevention of Adverse Drug Effects

Adverse drug effects in animals can be minimised but not altogether eliminated by observing the following points:

a. Employ drugs only when necessary.
b. Employ veterinary drugs which have been proven relatively safe in animals.
c. Avoid using human drugs for which animal safety data are not available.
d. Choose a drug that is safest among the available alternative drugs.
e. Use appropriate dose, route and frequency of drug based on the species involved.
f. Assess the benefits versus the risks of drug therapy and treat the patient accordingly.
g. Avoid polypharmacy (use of many drug simultaneously) or fixed-dose drug combinations.
h. Determine whether the patient already receiving a drug which might interact with the one proposed.
i. Elicit and take into consideration previous history of drug reaction.
j. Inform the customer about the possible adverse drug effects so that he can seek help in emergency and the drug can be withdrawn or dosage can be modified at an early stage.
k. Carry out appropriate laboratory monitoring, if required (e.g. prothrombin time with warfarin).

TREATMENT OF POISONING

Poisoning or toxicosis is a state that results from exposure to a poison. Poisoning may also result from inadvertent large doses of drugs because almost all chemicals possess inherent ability to produce toxic effects at different doses. Poisoning occurs infrequently, but it is often life threatening. So it needs to be managed immediately to save the life of intoxicated animal. Specific antidotes like receptor antagonists or chelating agents are available for only selected poisons and drugs (see for detail Chapter 76); for vast majority of drugs and chemicals, specific treatments are not available and symptomatic therapy that supports vital functions is the only approach.

Treatment of poisoning is based on three main principles: prevention of further absorption of poison, alteration of distribution and action of poison, and hastening elimination of poison.

I. Prevention of Further Absorption of Poison

Absorption of a poison in body can be prevented or decreased by using several non-specific (general) and specific agents/ measures. This approach helps to limit or delay the accumulation of toxic levels of a poison in the body.

Non-specific treatment

Non-specific agents/measures are commonly used in the treatment of poisoning in both human and veterinary practices. These agents/measures are generally considered safe, but their application depends on the route of exposure, type of toxicant and species involved.

1. Emesis

Emesis or vomiting is useful in recently ingested (within 2-3 hours) poisons in monogastric animals which are capable of vomiting. Emesis empties the stomach and decreases further absorption of poison. Emesis is indicated after ingestion of many drugs and poisons, but is contraindicated if a corrosive poison or petroleum distillate has been engulfed. It is also contraindicated in unconscious and comatose animals, severe respiratory distress or severe convulsive seizures (unless controlled) or where animal has ingested some foreign bodies or sharp-edged objects. Emesis can be induced in animals by using either locally acting (e.g. ipecacuanha syrup, hydrogen peroxide solution and hypertonic sodium chloride solution) or centrally acting (e.g. apomorphine and xylazine) emetics. The pharmacology and dosage of emetics are given in Chapter 44.

2. Gastric lavage

Gastric lavage is performed by inserting a tube into the stomach and washing it (stomach) with water or a solution. The tube is inserted to a length equivalent to the distance from the tip of nose to the last rib. After the stomach tube is put in place, the head is lowered to a 30° angle and the lavage fluid is first flushed into stomach at rate of 10 ml/kg body weight and then removed. Administration of lavage fluid must be gentle, preferably by gravity flow, to avoid injury to stomach. The process of pumping out of stomach fluid should preferably be done by suction pressure. The whole process is repeated several times until the recovered fluid is clear. Gastric lavage is a rapid and useful way of removing the poison from the stomach and is preferred when the emesis is refused, contraindicated or when collection of gastric material is required. The contraindications to this procedure are the same as for emesis, but unlike emesis gastric lavage can be performed in an unconscious or anaesthetised animal. An endotracheal tube must be placed to prevent aspiration of stomach contents. Lavage is useful in small animals for as long as 4 to 6 hours after ingestion of a poison. Commonly used agents, depending on the condition of patient or type of poison, are tepid water, isotonic sodium chloride solution (0.9%), potassium permanganate solution (1:2000), weak solution of iodine (1:250 of 5%), tannic acid solution and sodium bicarbonate solution.

In ruminants, a large hose inserted in the rumen is used to introduce water gently until the abdomen expands slightly. The stomach tube is then lowered and allowed to discharge as much fluid as possible. This can be repeated several times.

Enterogastric lavage: Enterogastric lavage (also called 'through and through enema') is a combination of gastric lavage and a retrograde high enema given with gastric tube and entrotracheal tube still in place. Enema fluid is administered gently until fluid from the gastric fluid flows, indicating nearly complete evacuation of the gastrointestinal tract. As enema fluid reaches the upper intestines, spontaneous vomiting may occur.

3. Adsorption therapy

Adsorption is the physical binding of a poison to an unabsorbable carrier, which is eliminated in faeces. Activated charcoal is usually considered the adsorbent of choice when poisoning is suspected. It effectively adsorbs a wide variety of drugs and chemicals (especially large non-polar molecules) and prevents their absorption and toxicity. Activated charcoal also can interrupt the enterohepatic circulation of poisons and enhance the net rate of diffusion of the chemical from systemic circulation/ bile into the gastrointestinal tract. Activated charcoal, however, poorly adsorbs some chemicals including caustics, corrosives and mineral acids. Ionized substances are also less firmly bound to activated charcoal than non-polar substances. Activated charcoal can be administered following emesis or gastric lavage, or when these measures are contraindicated. The effectiveness of charcoal is dependent on the time since ingestion and on the dose of charcoal (suitable adsorption is attained if charcoal : drug ratio is about 10 : 1). Activated charcoal can be given at a dose rate of 1-2.5 g/kg body weight in a slurry in which 1 g of charcoal is suspended in 5 ml of water. Charcoal is usually administered with a cathartic like sodium sulphate or sorbitol. This combination hastens removal of the poison-charcoal complex and helps to prevent constipation that can occur from charcoal. Care should be taken while administering specific antidotes by oral route because charcoal also may adsorb and decrease their effectiveness. The detailed pharmacology of activated charcoal is given in Chapter 43.

Universal antidote, a formulation initially synthesised for treatment of most poisonings, consists of 2 parts activated charcoal/burnt bread (for adsorption), 1 part tannic acid/strong tea (for precipitation) and 1 part magnesium oxide/milk of magnesia (for catharsis). Universal antidote is rarely used nowadays because neither it is universal nor an antidote to poisons. It is considered very less effective than the activated charcoal as different ingredients present in it interfere with each other's action and neutralise the overall efficacy. Egg white, milk and other demulcents also act as adsorbents but their efficacy is much lower than activated charcoal. Colestyramine has also been tried, but it has been found effective against only a limited number of drugs like cardiac glycosides.

4. Cleansing of skin or hair

If a toxicant has been spilled or applied over the skin or hair, then animal should be gently washed several times using water, mild shampoo or liquid dishwashing detergent. Greasy topical medications or solvents should be avoided as these may do more harm than good either by acting as vehicles to enhance absorption or by dispersing the toxicant and increasing surface area for absorption. Epidermal structures like wings, nails, claws, feathers, or fur should be cleaned thoroughly, paying particular attention to areas such as ears, between toes, etc. The cleaning should be undertaken quickly to avoid licking and ingestion of the toxicant, and to limit cutaneous absorption. Long and matted hair may be clipped, if necessary, for complete removal of toxicant residues. Persons washing the animals should wear gloves and other protective garments to avoid exposure to the toxicant.

5. Cleansing of eyes

For toxic ocular exposure, immediate flushing of eyes is advised. The eyes with lids wide open are gently flushed numerous times (20-30) with body temperature tap water, lactated Ringer's solution or normal saline solution to enhance the decontamination. Following adequate irrigation, animal should be examined for chemical burns to eye and, if found, treated with lubricant ointments to protect the damaged area.

6. Gastrotomy or Rumenotomy

Manual gastric emptying after emergency gastrotomy or rumenotomy may be necessary in situations which are refractory to emesis, lavage or adsorption therapy. Toxic metals (e.g. lead and zinc), indigestible materials (e.g. plastic and polyurethane foam), or toxic oils and tars are indications for surgical evacuation.

Specific Treatment

Antidotes can selectively interact with some poisons to chemically neutralise them or to form complexes, which are poorly absorbable. For example, silver nitrate poisoning can be treated with sodium chloride to form silver chloride that is less toxic. Similarly, sodium formaldehyde sulphoxylate can convert mercuric ion to the less soluble metallic mercury, and sodium bicarbonate can covert ferrous iron to ferrous carbonate that is poorly absorbed. Deferoxamine may trap gastrointestinal iron to form iron-deferoxamine complex, which is then excreted in the faeces. Likewise various neutralising agents (e.g. acids and alkalis), chelating agents (e.g. dimercaprol and calcium EDTA) and precipitating agents (e.g. tannic acid and potassium permanganate) have been recommended for various poisons. However, this technique is less used nowadays because efficacy and usefulness of many agents are doubtful and there is lot of controversy about their therapeutic applications.

II. Alteration of Distribution and Action of Poisons

Alteration of Distribution of Poisons

Some agents alter the distribution of poisons to their target sites. However, relatively few poisons are neutralised in this way. This approach includes some non-specific and specific measures/agents.

Non-Specific Treatment

1. Ion-trapping

The basic principle of ion-trapping is that ionized compounds do not readily traverse cell membranes and are, therefore, not distributed to target site and subsequently not re-absorbed by the renal tubules. The ion-trapping is more useful for enhancing the excretion of poisons then altering their distribution because the urine pH can be changed more easily as compared to the blood pH.

2. Alternate binding sites

Distribution can be affected by providing an alternate binding site to the poisons. For example, infusion of albumin provides a binding site to some poisons. This alternate binding site decreases plasma concentration of free form of poisons and, thus, their availability at target sites.

Specific Treatment

Antidotes can form complexes with poisons making them unavailable to cross cell membranes or to interact with their

target receptors. To be effective, the poison-complex formed must be both inactive and stable until excreted from the body. For example, protamine sulphate neutralises heparin and antivenoms inactivate venoms by forming non-toxic complexes. Similarly, lead toxicosis is treated mainly by administering calcium disodium edentate, which chelates the circulating and tissue lead in a non-toxic complex form and decreases its concentration at the target sites.

Alteration of Action of Poisons

Many agents alter action of poisons by reducing the sensitivity of individuals to poisoning mainly by increasing the threshold to poisoning. This can be achieved either by directly antagonising the effect of poisons or by providing appropriate emergency supportive and symptomatic therapy to the patient. In such cases, more concentration of poisons is required to produce toxic effects.

Non-Specific Treatment

Appropriate emergency supportive and symptomatic therapy is often the most valuable part of early treatment for poisoning. It provides clinical support to the vital functions of body and helps to correct the offending symptoms to give relief and stabilise condition of the animal. The type of supportive therapy required depends on the condition of animal and type of poison.

1. Treatment of respiratory depression

In case of respiratory depression or failure, a patent airway should be established with a cuffed endotracheal tube and adequate ventilation maintained by artificial respiration, if needed. Respiratory stimulants (e.g. doxapram) should be used only if necessary.

2. Control of shock

Shock may occur due to poisons causing excessive fluid loss, vomiting, diarrhoea, blood loss or cadiomyopathy. In poisonings, adequate circulation and perfusion must be ensured. Use of plasma volume expander, fluid therapy or whole blood administration may be followed. Use of excess fluid should be avoided once shock and hypovolaemia are stabilized, especially in conditions susceptible to pulmonary oedema or cerebral oedema.

3. Monitoring of cardiac disturbances

Continuous cardiac monitoring for arrhythmias is essential in comatose patients and patients with cardiotoxic drug exposure. In case of cardiac arrhythmia, appropriate antiarrhythmic drugs should be employed. When antiarrhythmic drugs are used, the therapy should be specific for the type of arrhythmia present and the species involved.

4. Control of acid-base disturbance and electrolytes imbalance

Provision of adequate hydration, acid-base and electrolyte balance are all necessary for normal cardiovascular functions. These should be monitored closely and appropriate fluid and electrolyte therapy administered (see for detail Chapter 40). Metabolic acidosis is the most common drug associated acid-base imbalance. It can initially be treated with either sodium bicarbonate or sodium lactate.

5. Treatment of CNS dysfunctions

Central nervous system stimulation or depression is a common result of poisoning. Use of appropriate drugs to counteract it may be essential to mitigate the symptoms of poisoning. Seizures can be controlled by using CNS depressants (e.g. diazepam, phenobarbital and pentobarbital). Diazepam is the drug of choice for acute seizures of unknown aetiology. Phenobarbital is used if diazepam is ineffective or prolonged seizure control is needed; pentobarbital and other CNS depressants can also be used. In case of CNS depression, analeptics such as doxapram (1-2 mg/kg, IV) have been recommended, especially when respiratory depression is more severe.

6. Treatment of thermoregulatory disturbances

Hypothermia or hyperthermia may occur during poisoning and should be treated promptly.

a. ***Hyperthermia :*** Hyperthermia often occurs with persistent seizures. Hyperthermia may also be a problem with toxicosis caused by uncouplers of oxidative phosphorylation (e.g. pentachlorophenol). Hyperthermia is treated with cold baths, ice packs or cooled intravenous fluids. Exercise or high ambient temperature generally aggravates hyperthermia, so the intoxicated animal and the surroundings should be kept calm and cool. Antipyretics are generally of little help in hyperthermia induced by drug.

b. ***Hypothermia :*** Hypothermia may occur during anaesthesia, heavy sedation or coma. Vital physiological processes are generally depressed when body temperature is sub-normal. Hypothermia may be prevented through use of blankets, warm water bottles and heating pads. Warming the surroundings of the animal is safer than directly applying heat to the body.

7. Maintenance of uresis

Adequate renal blood flow can be maintained by using osmotic diuretics like mannitol (1-2 g/kg, IV). Mannitol also helps to reduce cerebral oedema in poisoned animals.

Specific Treatment

Use of specific antidotes to drugs is most effective in some poisoning cases. Antidotes produce their effect in a variety of ways including chelation, enzyme inhibition or receptor antagonism. Antidotes are generally administered after exposure to poisons often in response to clinical toxicoses. Some antidotes may be toxic, particularly when used in higher dose or for longer duration. As many antidotes are specific to toxicants, their use in absence of the particular drug or toxicant may produce their own adverse effects. Therefore, correct diagnosis is important prior to the use of antidote. General decontamination and use of supportive therapy often help in the effectiveness of antidotal treatment. Examples of some common drugs and poisons and their antidotes are listed in Table 8.3.

III. Hastening Elimination of Poisons

Some therapeutic agents/measures enhance elimination of poisons from body either by altering their metabolism or by enhancing their excretion. These agents, therefore, rapidly decrease toxic levels of poisons in the animal body.

Table 8.3: List of some common poisons and their antidotes.

Sr. No.	Drug/Toxicant	Antidote/Drug therapy	Product/Mechanism of action
1.	Antimony	Dimercaprol, penicillamine	Chelation
2.	Arsenic	Dimercaprol, penicillamine	Chelation
3.	Atropine	Physostigmine	Receptor antagonism
4.	Benzodiazepines (e.g. diazepam)	Flumazenil	Receptor antagonism
5.	Carbamate insecticides	Atropine	Receptor antagonism
6.	Curare	Neostigmine	Antagonism
7.	Cyanide	Sodium nitrite, sodium thiosulphate	Alternate pathway
8.	Ethylene glycol	Ethanol	Alternate pathway
9.	5-Fluorouracil	Thymidine	Alternate pathway
10.	Iron	Deferoxamine	Chelation
11.	Lead	Dimercaprol, calcium disodium EDTA	Chelation
12.	Nitrite	Methylene blue	Alternate pathway
13.	Opioids (e.g. morphine)	Naloxone	Receptor antagonism
14.	Organophosphorus insecticides	Atropine	Receptor antagonism
15.	Paracetamol	N-Acetylcysteine	Alternate pathway
16.	Warfarin	Vitamin K	Antagonism

Non-Specific Treatment

1. Catharsis/Purgation

Catharsis is indicated for removal of unabsorbed poisons especially those which have passed into intestine. Use of cathartics in the management of orally ingested poisons is only of minor importance as they produce loss of fluid and electrolyte and, thus, may cause dehydration and electrolyte imbalance. Preferred agents for most poisonings are osmotic saline purgatives (e.g. magnesium sulphate and sodium sulphate) and sorbitol. Magnesium based cathartics may cause hypermagnesaemia under some conditions and should not be administered in poisoning with substances which decrease GI transit time or in case of renal insufficiency. These agents may be used with or without activated charcoal. If used with charcoal, then cathartic may be given 30 min after administration of activated charcoal. Bulk purgatives which do not contain sugar are preferred for toxicants or physical agents (e.g. lead objects or fibre glass insulation) those need safe passage through GI tract. Use of oily cathartics including mineral oil (except in phosphorus intoxication), vegetable oils and irritant purgatives are not recommended in poisoning as they often increase the absorption of toxicant. Cathartic is not recommended if animal has diarrhoea. Inadvertent and indiscriminate use of cathartics may produce dehydration, hypernatraemia, hypermagnesaemia, hyperkalaemia, etc. The pharmacology and dosage of cathartics are given in Chapter 43.

2. Ion-trapping

Alteration of urine pH is a convenient and useful method of preventing renal reabsorption of poisons which undergo glomerular filtration and passive tubular reabsorption. The ion-trapping is carried out with the help of an appropriate urinary acidifier or alkalizer and is clinically useful for enhancing the excretion of many weakly acidic or basic compounds from body. The Henderson-Hasselbalch equation provides the basis for ion-trapping of ionised compounds. Generally acidic agents (e.g. aspirin, paracetamol and barbiturates) are more effectively ionised and excreted in alkaline urine and basic agents (e.g. amfetamine and most alkaloids) are more effectively ionised and more readily excreted in acidified urine. Ion-trapping therapy is most suitable in acid-base imbalance conditions.

3. Dialysis

Dialysis may be described as the movement of an agent across a semi-permeable membrane. Haemodialysis, peritoneal dialysis and haemoperfusion are important types of dialysis, but these are possible only in sophisticated hospitals. Haemodialysis involves passage of blood from an artery through dialysis membrane which is then returned to body via a vein. In peritoneal dialysis, an essentially isotonic solution is introduced into the peritoneal cavity and later withdrawn. Here, the peritoneum itself serves as the dialysis membrane. Haemoperfusion involves passage through charcoal or some other chambers. Dialysis is useful when patient is in comatose condition, or in situations of renal failure, anuria, hypotension, fluid and electrolytes imbalance, and hepatic failure. Dialysis should also be considered if large amounts of drug are present in the body. Peritoneal dialysis procedure has limited value for the management of some poisonings as fluid needs frequent changes. Dialysis is useful in intoxication by digitoxin, methanol, lithium, phenobarbital, etc.

4. Diuresis

Forced diuresis may be defined as the promotion of rapid renal filtration of a compound by using certain therapeutic agents. Forced diuresis may be useful for some, but not all poisons because for it a compound must have to be present in high concentration in an unbound form. Moreover, a substantial portion of filtered poison is often reabsorbed by the renal tubules. Forced diuresis may be considered useful in certain conditions such as in toxicosis with serious clinical signs (e.g. severe hypotension, coma and arrhythmia), when potentially lethal dose of a compound has been ingested, or when there is progressive deterioration of the condition of animal in face of intensive therapy. Forced diuresis is also useful in oedema due to complications of poisoning i.e. heart failure, ascites from liver failure, pulmonary oedema, acute renal failure, etc. Forced diuresis in most toxicity cases is achieved either by use of diuretics (e.g. mannitol and furosemide) or by

intravenous administration of parenteral fluids (e.g. 5%, 10% or 50% dextrose). In large animals, it can be more practical to pump large volumes of water or non-sterile electrolyte solution into the rumen or stomach, often in conjunction with a diuretic. Excessive administration of IV fluid should be avoided as it may result in complications including cerebral oedema, pulmonary oedema and disturbance in acid-base or electrolytes status.

5. Biotransformation

Many drugs are detoxified by biotransformation before excretion from the body. Acceleration of biotransformation through sufficient availability of endogenous co-substrates for conjugation may enhance rate of elimination of poisons. Although induction of oxidative enzymes is too slow (days) to be valuable in the treatment of acute poisoning by most chemicals, enhancing availability of endogenous conjugating substances is useful in certain poisonings. For example, paracetamol induced liver toxicity can be prevented by maintenance of adequate concentration of glutathione, and this can be accomplished by administration of N-acetylcysteine. Similarly, detoxification of cyanide by conversion to thiocyanate can be accelerated by administration of sodium thiosulphate.

Specific treatment

Certain drugs selectively enhance excretion of some poisons from the body. For example, chloride accelerates renal excretion of bromide, and tetrathiomolybdate enhances biliary excretion of copper. Sulphate limits molybdenum concentration in body both by reducing its intestinal absorption and by increasing its urinary excretion. Some chelating agents (e.g. dimercaprol, D-penicillamine and calcium disodium edetate) form complex with metals like lead, copper and arsenic and enhance their excretion from body.

MANAGEMENT OF POISONING

Poisoning is always an emergency and needs to be managed immediately. In addition to treatment of poisoning as discussed above, certain management practices also help in the therapy and should be used as applicable.

a. When poisoning has occurred or is occurring, further exposure to the toxicant should be avoided. In case of livestock, changing pastures or feedlots may prevent further exposure. Shifting of pets from area of exposure to another site may also be helpful. Changing of feed and water source prevents further potential ingestion of toxicant.

b. Intoxicated animal should be kept at a place which is free from excessive noise and other disturbances. Convulsive seizures usually aggravate with external stimuli.

c. If animal has been fastened with a chain or a rope, its removal is required otherwise animal may strangulate itself or may get hurt.

d. Vicinity of intoxicated animal should be kept free of sharp objects and obstacles. This helps in decreasing injury to the intoxicated animal.

e. Head of a recumbent animal should be kept at a lower level than rest of the body. This will prevent aspiration of regurgitated material.

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CHAPTER 9 DISCOVERY AND DEVELOPMENT OF NEW DRUGS

- Drug discovery
- Drug development
  - Pre-development stage
  - Development stage
    - Pre-clinical development
    - Clinical development
  - Orphan drugs
- Recombinant biotechnology
  - Fundamentals of biotechnology
  - Fundamental genetic processes
  - Molecular biological tools

The development of new drugs is a costly, time consuming, and complicated process. It involves coordination of many scientific experts from varied disciplines like organic and analytical chemists, pharmacists, microbiologists, pathologists, toxicologists, pharmacologists, veterinarians and many others. Majority of drugs those are first shown to possess pharmacological activity in laboratory animals are subsequently rejected before they reach target clinical trials. Even the drugs which survive the initial stages may not be marketed due to failure at subsequent stages. It is estimated that out of every 5,000 new compounds identified during the discovery process, only five are considered safe for testing after preclinical evaluations. After three to six years of further clinical testing in animals, only one of these compounds is ultimately approved as a marketed drug for treatment. The entire process of discovering and bringing one new drug to the market typically costs a pharmaceutical or biotechnology company nearly $800 million to $1.7 billion and takes an average of 10 to 12 years. This cost may not be recovered by the pharmaceutical firm, if the drug fails in the market due to identification of some post-marketing adverse effects. All these factors make discovery and development of truly novel products very expensive; therefore, unique products in animal health marketplace are becoming less frequent. Nevertheless, new veterinary products are being developed and introduced into market every year and gaining success in the field of veterinary therapeutics.

DRUG DISCOVERY

Drug discovery is the process by which new drugs are discovered and/or designed for some therapeutic efficacy. Once a compound has been identified for therapeutic value in discovery phase, it will begin the process of drug development. In the past, most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. However with advancement in the technologies, new approaches like target specific molecular designing are increasingly used for drug discovery. Despite advances in technology and understanding of biological systems at molecular levels, drug discovery is still a lengthy, expensive, difficult and inefficient process with low rate of new therapeutic discovery. The major pharmaceutical establishments, committed to continuous drug research and development (R & D), discover new drugs mainly by following procedures: molecular designing, molecular manipulation, bioprospecting, random screening, serendipity, active metabolite and genetic engineering.

1. Molecular designing

Molecular designing is the structuring or designing of a new substance to fulfil specific biological tasks. In recent times, molecular designing is considered the most rational form of drug development and research. In this technique, scientists initially identify a target, generally a naturally existing cellular or molecular structure involved in some disease process and then subsequently design molecules which can interact with this target and might be helpful in treatment of that disease. In the modern era of receptors, drug molecules can be designed with affinity for specific receptor types and subtypes to produce accurate pharmacological effects. The other important targets currently selected for drug discovery efforts are newly discovered proteins and ion channels. With the advent of computer technology and microelectronics, new methods are being applied to molecular designing. Computers are now used to store large number of chemical structures from which new molecules can be easily designed. Also the computer's databank supplies a correlation between a given molecular structure and its likely effects. Additionally, computers visualise macromolecules such as receptors and can design a matching drug molecule. This elective designing of a drug on the electronic drawing board is called molecular modelling. Example of drug synthesised by molecular designing is allopurinol, an analogue of xanthine.

2. Molecular manipulation

Molecular manipulation or molecular roulette is the chemical modification of a known molecule. Molecular modification is widely used in discovery of new drugs or screening of natural products for biological activity and subsequent semi-synthetic production. This procedure allows the organic chemists to use natural drugs

from plant or animal source as starting material in the creation of slightly different molecule or sometimes the synthesis of totally new agents. Such agents may have superior pharmacological properties and fewer side effects. Altered patterns of absorption, distribution or elimination may be achieved with resultant improvements in therapeutic efficacy and safety. For examples, several semi-synthetic opioids have been prepared by chemical modification of morphine. The thiazides and oral hypoglycaemic sulphonylureas represent slight modifications of an old antibacterial drug sulphanilamide. Using molecular manipulation of natural antibiotics, many semi-synthetic penicillins and cephalosporins have been similarly developed.

3. Random screening

Screening is the evaluation of a drug for different types of biological activity. In this procedure, a chemical substance is subjected to a number of tests designed to determine its different types of pharmacological effects. Such tests may include studies on animal behaviour, isolated tissue preparations, intact animals and sometimes animal models of diseases. Discovery of new drugs by screening process is a difficult and complicated task because it often involves major costs and many years of extensive study. It has been estimated that only one in every 4000 to 5000 new chemical substance screened is marketed for clinical use. Despite all the odds, testing large numbers of compounds to see if they produce an appropriate biochemical or cellular effect is usually one of the first steps in the drug-discovery pathway. The antibacterial drug tetracycline was discovered through the procedure of random screening.

4. Bioprospecting

Bioprospecting is the exploration of biological diversity for molecules to be used as drugs or other commercial products. Bioprospecting has been an important procedure of discovering new drugs for centuries. In the early stages bioprospecting largely centred on the plants from the forest ecosystem, but in recent times various other forms of biodiversity like insects, algae and microorganisms have been explored to see if they harbour cure for a human or animal disease. Bioprospecting is a very costly procedure, but it has yielded a large number of drugs including the anti-inflammatory aspirin, discovered a century ago from a willow tree, and the anti-oestrogen tamoxifen, derived in the last decade from the bark of a yew. Discovery of several life-saving drugs including anti-neoplastic drugs (e.g. vinblastine, taxol, topotecan and etoposide) in recent past has renewed the interest of pharmaceutical industries in bioprospecting.

5. Serendipity

Serendipity means accidental discovery or finding of one thing while looking for something else. Serendipity has led to introduction of many useful drugs in the therapeutics. Sometimes new uses are found for old drugs and the side effects find therapeutic applications. For example, Alexander Fleming accidently discovered penicillin while observing mould contaminations in one of his cultures. The serendipitous discovery of some useful therapeutic effects of drugs include cardiac antiarrhythmic effect of lidocaine while examining its local anaesthetic action, tranquilizing effect of chlorpromazine while observing its antihistaminic effect and anti-baldness effect of minoxidil while studying its anti-hypertensive effect.

6. Active metabolites

Many drugs have been found to convert to one or more active metabolites in body, which have stronger action and possess therapeutic advantages over the parent compounds. Some of these active metabolites were discovered after the marketing of parent drugs and were later on synthesised and marketed as newer therapeutic agents. These newer drugs show pharmacological activity similar to their parent compounds, but possess significant advantages in terms of better efficacy, potency or pharmacokinetics, or lesser adverse effects. The role of active metabolites in the drug development gained impetus in late 1930s with the discovery of the synthetic azo dye prontosil's metabolism to antibacterial agent sulphanilamide in the body. Sulphanilamide was later on synthesised and marketed as a drug and became very popular antibacterial agent. Other drugs discovered on the basis of drug metabolism include paracetamol, an active metabolite of phenacetin, and oxazepam, an active metabolite of chlordiazepoxide.

7. Genetic engineering/Recombinant DNA biotechnology

Genetic engineering or recombinant DNA biotechnology means manipulation of genetic elements which result in alterations to proteins or changes in pattern of proteins expression. With genetic engineering, a number of new and more selective drugs can be produced. Genetic engineering is useful because it allows production of a wide range of biological substances including many immunologically active factors in abundant quantity. In human and veterinary medicines, genetic engineering has yielded useful recombinant vaccines for the prevention of viral, bacterial and parasitic diseases. Examples of recombinant drugs include recombinant human erythropoietin (rhEPO), recombinant human insulin, and recombinant bovine somatotropin (bST). The use of genetic engineering technique has led to production of several recombinant vaccines like recombinant pseudorabies vaccine, recombinant feline rabies vaccine and recombinant canine distemper virus vaccine. The molecular biological tools and procedures of recombinant biotechnology have been discussed separately in the present chapter.

DRUG DEVELOPMENT

Drug development is a broad term used to define the entire process of bringing a new drug to the market, once the candidate drug (often called new chemical entity) has been discovered or synthesized. The drug development is essentially required to establish the physicochemical properties, safety, pharmacokinetics, pharmacodynamics and therapeutic utility of the candidate drug so that it can be made available for wide-scale clinical use. The benefits of the drug are critically weighed against giving no treatment or against alternative forms of treatment. Many aspects of drug development are focused on satisfying the regulatory requirements of drug licensing authorities. Although animal testing in the research-based pharmaceutical industry has been reduced in recent years both for ethical and cost reasons, but still most drug development research involve animal based testing for the need of similarity in anatomy and physiology in different species. All studies must strictly adhere to Good Laboratory Practices (GLP) to be acceptable for submission to regulatory agencies. The entire drug development studies can be broadly divided into parts: pre-development and development stages.

A. Pre-Development Stage

Pre-development is a stage of research in which some basic research data are produced before the actual drug development stage starts. In this stage, new compounds showing a particular characteristic or useful pharmacological property are put through a battery of tests to establish its utility to carry out the more costly and detailed development phase. For instance, the candidate compound may undergo physico-chemical analysis, pharmacological studies and short-term toxicity testing to establish it's pharmacological and safety profile. Some of the important pre-developmental stage tests are given below.

1. Physico-chemical analysis

When a new compound has been discovered and isolated, it is analysed for the physico-chemical properties. The structure of new compound and its purity is determined and confirmed by the analytical chemist. Chemical showing a particular structure or physical characteristic considered of potential value is subjected to screening.

2. Screening

A pharmacological screen is a series of procedures, which determine whether or not the drug molecule possesses useful biological properties. In screening process, the pharmacologist administers a few doses of the compound over a wide range to a small number of mice or rats and then observes these animals for a few hours or days for biological effects. Careful observations at this stage can give clues to numerous types of pharmacodynamic effects. Even if the molecule is from a family of well-studied chemicals with common structural properties, screening is necessary to demonstrate unsuspected side effects of the drug or to reveal its undiscovered therapeutic activity.

3. Pharmacological profile

When a compound has shown activity upon screening, it is evaluated for its pharmacological profile. Usually it involves secondary and tertiary screening during which full spectrum of the agent's biological or pharmacological properties, and if possible its mechanism of action and metabolic fate are defined. The structure activity relationships (SAR) are also determined. Quantitative and qualitative bioassays are widely used for these procedures.

4. Short-term toxicity tests

Short-term toxicity tests are conducted to detect the possible toxic properties of the drug. These tests are useful to evaluate nature and degree of toxic symptoms and to determine the agent's margin of safety. The extensiveness of these tests is determined by the proposed clinical application and by the physical and chemical properties of the agent.

Once the potential usefulness of an agent has been determined by the pre-development stage tests, the agent may be considered for further exploration as a successful drug in the development stage. At this point, major costs to be involved in the development of a new drug should be considered and, if found suitable, the necessary patent application should be made. A patent permits the manufacturer to proceed unhindered by rival competition with R & D and marketing of the compound and serves as a form of compensation for costs incurred.

B. Development Stage

Once laboratory tests have indicated useful pharmacological activity and acceptable toxicity profile, the candidate drug enters the development stage. This development stage (Figure 9.1) is a high-risk enterprise because it involves considerable investment in time, revenue and labour. The data generated by development stage are usually used to support registration application and marketing. The development stage may be further divided into two sub-stages: pre-clinical development (on experimental animals) and clinical development (on target species).

Pre-clinical development

Pre-clinical development (Pre-clinical studies or non-clinical studies) is a stage of research that begins before clinical trials (testing in target species) can begin, and during which drug may undergo pharmaceutical, pharmacodynamic, pharmacokinetic and toxicity testing studies through animal testing. The main goals of pre-clinical studies are to determine a product's ultimate pharmacological and safety profile. These data also allow researchers to estimate a safe starting dose of the drug for clinical trials in target species. Based on pre-clinical trials, No Observable Effect Levels (NOEL) on drugs are established.

1. Pharmaceutical studies

In pharmaceutical studies, suitable formulations of drug are developed for laboratory and clinical trials. Various manufacturing processes and analytical methods for determination of drug concentrations are also developed. Studies regarding the stability and shelf life of the drug are undertaken. The candidate drug is further examined for its suitability to be made into capsules, tablets, aerosol, intramuscular or subcutaneous injectable, or intravenous formulations.

2. Pharmacological studies

The drug selected for development is subject to detailed pharmacodynamic and pharmacokinetic studies. In pharmacodynamic studies, effects of drug on various body organs and systems are evaluated. Tests are undertaken for study of dose-response relationships, efficacy, resistance, tolerance, drug-interactions, etc. Pharmacokinetics data establish the bioavailability of compound and determine its distribution in body. Such studies are also useful in estimating elimination half-life of the drug and in calculating the optimal dosage schedule to be used for clinical trials. These studies are supplemented by radiotracer techniques and precede comprehensive metabolism studies in both laboratory and target species.

3. Toxicity studies

The toxicity studies of a drug must be conducted in light of the purpose for which it is to be used, period over which it is to be used, and effective dosage to be used. While these tests can be done using *in vitro* methods (e.g. with isolated cells), many tests can only be made by using experimental animals, since it is only in an intact organism that the complex interplay of metabolism and drug exposure on toxicity can be examined. Use of animals in toxicity studies also facilitates detection of infrequently occurring adverse effects as well as statistical exploration of data. The choice of species generally varies based on which will give the best correlation to the target species trials. Particularly sensitive species may be needed to investigate special long-term toxicity effects such as teratogenicity or carcinogenicity.

a. *Acute toxicity:* Acute toxicity study reveals the type and extent of adverse effects a compound produces when administered as a single increased dose or multiple doses during a 24-hour period. This study provides the median lethal dose (LD_{50}) and also helps to determine the therapeutic index. Acute toxicity is mainly carried out in large numbers of well-known strains of at least two mammalian species using equal numbers of both sexes. Rodents such as mouse, rat and hamster are most commonly used. One rodent species may be replaced, if appropriate, by an animal species for which the medicinal product is intended. Dose levels should be chosen to demonstrate the entire spectrum of toxicity. At least two different routes of administration are normally used out of which one should be identical with or similar to that proposed for use in target species.

b. ***Subacute toxicity:*** It is the study of repeated exposure of a drug and its effects for 30 days or less. This study reveals various physiological or pathological changes induced by multiple dosing with the active substance and determines how these changes are related to dosage. This is especially important when a drug is intended to be used repeatedly in the target animal. The drug is usually administered orally or applied dermally depending on its intended use. Species differences between the laboratory species (e.g. rat) and the final target species must be considered.

c. ***Chronic toxicity:*** In chronic toxicity study, the drug is administered for several months (minimum 3 months) and its effects are studied. Generally two species of animals, one rodent (e.g. rat) and one non-rodent (e.g. dog) are chosen for the study and drug is administered for 2 years. The 2-year period is chosen because this accounts for the life span of the rat. At the end of the drug exposure period, animals are sacrificed and extensive biochemical and histopathological examinations are made. In addition, special tests are conducted to access potential for reproductive, teratogenic, mutagenic, or carcinogenic activity.

i. *Teratogenic studies:* Since the discovery of teratogenic effects of thalidomide in children in the late 1950s, it has become mandatory to test all new drugs for the possible adverse effects on foetus. Such studies are carried out in two animal species in which different dose levels of drug are given at various stages of pregnancy. Foetuses are carefully examined for visceral and skeletal deformities, and the number of live and dead foetuses is recorded. The rabbit is the experimental animal of choice because of the ease with which teratogenic effects can be induced in this species.

ii. *Carcinogenic studies:* Carcinogenic studies are conducted particularly when the drug has a close chemical similarity to a known carcinogen or co-carcinogen. In this study, the candidate drug is administered to test species for a longer period (2 years) and animals are carefully observed for development of tumours. An increased incidence of tumours in treated animals than in untreated animals, the development of tumours earlier than in control animals, and the occurrence of types of tumours usually not seen in untreated con animals are the criteria on which a substance ma declared carcinogenic.

iii. *Mutagenic studies:* Mutagenicity tests are perform evaluate the possible mutagenic activity of the chemical. The short-term tests generally used inclu bacterial gene mutation assay (e.g. Ames *Salmon* assay) and a mammalian cell gene mutation a Bacterial mutagenicity tests are performed beca extensive data exist on the correlation between resu such tests and carcinogenicity as determined by lo term whole animal studies.

iv. *Fertility and reproductive studies:* These studies carried out mainly to evaluate the possible adverse ef of the drug on the fertility and reproductive performar In this study, rats are treated with the new drug b and after the mating period and effects on early and l stages of embryonic and foetal development are stu Three generation reproductive study may also performed to evaluate the adverse effects of dru next generations.

Clinical development

Clinical development begins when pre-clinical data h established the apparent safety and efficacy of the candid drug. The primary aim of the clinical development stage is to m the candidate drug suitable for therapeutic use in target spec and help in its registration and marketing. The clinical developm stage usually comprises of clinical studies or trials.

1. Clinical studies

Clinical studies (Clinical trials, field trials, target animal stu include large-scale field trials of the new drug in the target speci The objectives of the animal field trials are to ascertain therapeutic effect of drug, to specify the indications a contraindications of drug according to the species and age, identify the side effects of drug in the target species, and ensure the safety of drug under general conditions of use. Fi trials are conducted at the proposed dose rate and at three to fo times that level. Field trials may be operated at three levels investigations: Phases I, II and III.

i. ***Phase I: Clinical pharmacological evaluation:*** Pha I trials are conducted in small number (usually 2 100) of healthy target animals spread over a period several months. The study determines whether the ta animals display the same/similar biological response observed in laboratory species used in pre-clin testing. The study identifies the metabolic ar pharmacological effects in target species which ma useful in therapy. The purpose of the phase I studi is also to determine the side effects associated w increasing doses and, if possible, gain early evidenc on the effectiveness of the drug.

ii. ***Phase II: Controlled clinical evaluation:*** Phase II tri are carried out in relatively large number (seve hundred) of target animals spread over a period of seve months to 2 years. The phase II trials are considered a extension of Phase I trials, but these are stric controlled, closely monitored and mainly aimed t ascertain the safety and efficacy of the new drug

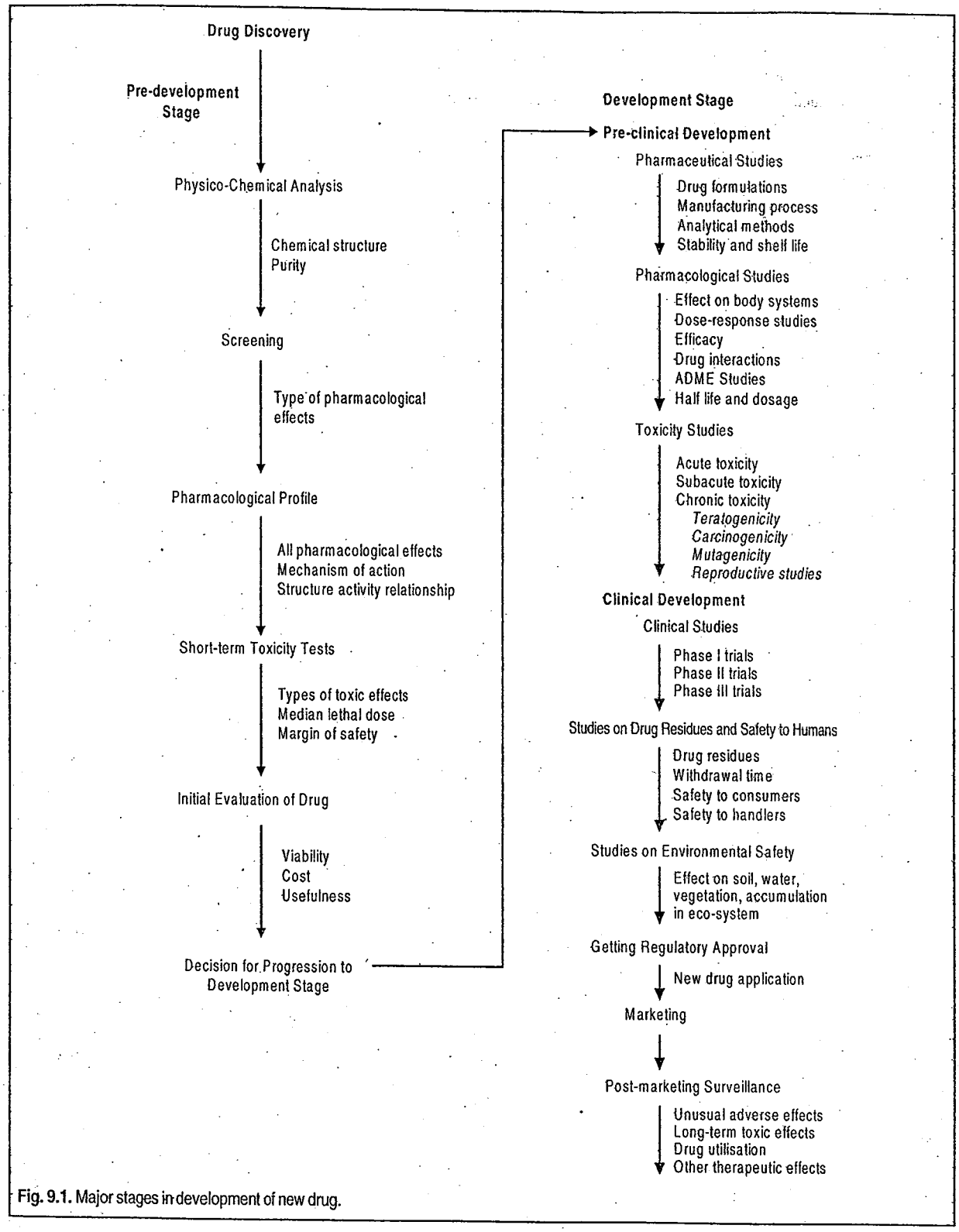


Fig. 9.1. Major stages in development of new drug.

target species. Studies are conducted to obtain some preliminary data on the effectiveness of the drug for a particular indication or indications in animals with the disease or condition. Drug toxicity and therapeutic usefulness of the new drug are carefully recorded and evaluated.

iii. ***Phase III: Extended clinical evaluation:*** Phase III trials comprise a large-scale extensive clinical study designed to evaluate the effect of drug in target animals at different locations under varying conditions of housing and management. These are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase II, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug. Phase III studies also provide an adequate basis for extrapolating the results of the clinical trials to the general animal population and

transmitting that information in the drug labeling. These trials thus indicate reliability of the drug under field conditions of use. The phase III studies usually include several hundred to several thousand animals and are spread over a period of 1 to 4 years or more.

Problems of clinical trials: The three phases of field trials are generally conducted over several years at various geographical locations, seasons and management conditions, and are repeated several times to provide useful and reliable data. Therefore, the element of bias (to have a preconceived idea about the result) is also high in such trials. To eliminate both subjective and environmental bias, some steps are essentially required:

i. ***Randomisation:*** In field trial, all animals should be selected at random. The frequent practice is to block animals into different groups based on breed, sex, weight, age or origin and then select them randomly into a number of comparable groups. This system of randomisation avoids subject-characteristics playing a role in assignment to a particular group.

ii. ***Control animals:*** Field trials should always be performed using control animals. Control animals are useful in eliminating false positive (Type I error) or false negative (Type II error) conclusions. Type I error means that the trial has shown a biological (therapeutic/toxicological) effect, while none actually exists. Type II error means that the trial has found no effect, while actually it exists. Such errors can also be minimised or eliminated by subjecting the trial data to suitable analysis.

iii. ***Blind design:*** It is a clinical trial (design) in which the investigator does not know (is blind) which are the control and treated groups. The drug and vehicle both look alike and are coded, and the animals are numbered. The investigator, therefore, remains unaware of the type of treatment he is administering to the animals and his recordings are not due to any bias. (In case of experiments on human subjects, the terms single blind or double blind designs are used. In single blind trials, either the investigator or the patient does not know which treatment is being administered until the final analysis of the results. In double blind trials, both the investigator and the patient are unaware of the type of treatment given).

iv. ***Placebo:*** It is a pseudo-drug that is identical in appearance with the drug being tested, but is pharmacologically inert. Placebo is widely used in experimental research where comparison of the active drug to a placebo distinguishes the pharmacological effect from the non-specific effect. Placebo can also be used for blind trial to minimise chances of bias. In human medicine, the use of placebo constitutes a positive means of psychological therapy to satisfy the patients' demand for medicine. Experimental evidences have indicated that many chronic diseases of humans, which have a substantial psychogenic element, respond favourably to the placebos.

2. Studies of drug residues and safety to humans

The candidate drug should not only be safe to the animals receiving the drug, but also to the consumers of animal products and handlers of the compound. Therefore for veterinary drugs, testing may have to be done on the drug metabolites and their residual effect. From these data, drug withdrawal times and risk assessment for humans as consumers of animal products are laid down. Safety to humans is also examined in regard to actual contact with drugs during manufacturing and handling. On completion of these studies, precautions to protect animals, users and consumers are defined. Methods must also be developed and described by which the drug can be estimated in feed and in animal products.

3. Studies on environmental safety

A recent addition to the requirements imposed on the registration of a new drug is the determination of its effect on the environment. Tests must be designed to see the effect of drug on soil, water and animal or vegetable life. The drug must not adversely concentrate along food chains and must not accumulate in the environment.

4. Getting regulatory approval

When the comprehensive trial programme and all of the various experimental studies have been conducted and when the sponsor is satisfied about the safety and efficacy of the drug, an application, often called New Drug Application (NDA), is filed with the Drug Control Authority for product licensing and marketing authorization. The documentation required in an NDA is supposed to tell each and every detail of the tests conducted, including what happened during the clinical tests. If approved by the Drug Control Authority, the drug is marketed for general use.

5. Post-marketing surveillance

Post-marketing surveillance, sometimes called Phase IV of clinical trial, is the study made on patients by participating clinicians. The aim of Phase IV is to find out the safety profile in patients across the world and to determine certain unusual type of adverse reactions and toxic effects on long-term use of the drug. The study also determines the drug utilisation pattern, additional therapeutic effects, etc. This study continues till the drug is used.

Orphan Drugs

Orphan drugs are the pharmaceutical agents which have been designed specifically to treat rare medical conditions. These drugs are generally not developed into usable medicine because the cost involved in their manufacturing is very high and is not likely to be recovered by the developer due to limited number of patients. To tackle such situations, several countries have enacted special orphan drug legislations by providing tax incentives to pharmaceutical companies undertaking the development of such drugs in the interest of patients. The assignment of orphan status to a disease and to any drug developed to treat it is a matter of public policy in many countries. Orphan drugs generally follow the same regulatory development path as any other pharmaceutical product, in which testing focuses on pharmacokinetics and pharmacodynamics, dosing, stability, safety and efficacy. However, some statistical burdens (e.g. number of patients) are lessened in an effort to maintain development momentum.

RECOMBINANT BIOTECHNOLOGY

Recombinant biotechnology or genetic engineering may be defined as manipulation of genetic elements so as to produce

alterations in proteins or changes in patterns of proteins expression. More specifically, the term refers to techniques which are used to manipulate, move, recombine and propagate DNA. This biotechnology has led to an increased understanding of fundamental properties of genes and proteins and has provided detailed information for successful development of a number of important drugs. Innovative therapies for cancer, immunological disorders, inflammatory diseases, and others have occurred through increased availability and testing of new biological substances. It is expected that recombinant technology will have a tremendous impact on future medicine and will lead to new approaches for the diagnosis and treatment of many incurable diseases.

Fundamentals of Biotechnology

The deoxyribonucleic acid (DNA) contains the information that gives rise to the chemical and physical properties of living organisms. The DNAs isolated from different organisms have two strands in complementary double helical arrangement. This similarity among diverse kinds of organisms makes it possible to take genetic material from one species and recombine it with the genetic material from another species. Understanding how genetic elements are manipulated to produce recombinant drugs and vaccines requires familiarity with fundamental processes in the cell, and knowledge of molecular biological tools and procedures.

Fundamental Genetic Processes

The three major processes involved in the presentation and transmission of genetic information are replication, transcription and translation.

1. Replication

Replication is a process by which strands of DNA are accurately copied during cell division to form identical daughter molecules. Replication is an important cellular process because it allows transfer of genetic information from one generation to the next. During replication, two strands of a DNA double helix uncoil through action of several proteins and enzymes, and each strand acts as template for the formation of a new, complementary strand. Then each template strand and its complementary strand serve as the DNA in daughter cells. The net result of replication is two DNA molecules, each identical to the original double helix (Figure 9.2). Enzymes called DNA polymerases catalyse the DNA synthesis that occurs during the process of replication.

2. Transcription

Transcription is the process by which the genetic message in DNA is transcribed into the messenger RNA (m-RNA), to be carried to the ribosomes. Transcription is similar to replication in that DNA provides a template for construction of a new nucleic acid molecule, this time the ribonucleic acid (RNA). During transcription, a partially unwound section of DNA double helix is recognised by the enzyme RNA polymerase, which catalyses template directed synthesis of a pre-messenger RNA (with uracil, not thiamine, complementing adenine). Eukaryotic pre-mRNA is processed in the nucleus into mature m-RNA, which then travels to the cytoplasm where the genetic code is translated to form a specific protein. In prokaryotes, m-RNA is not post-transcriptionally processed because the DNA is transcribed directly into mature m-RNA. In fact in prokaryotes, the translation process often begins before transcription is complete.

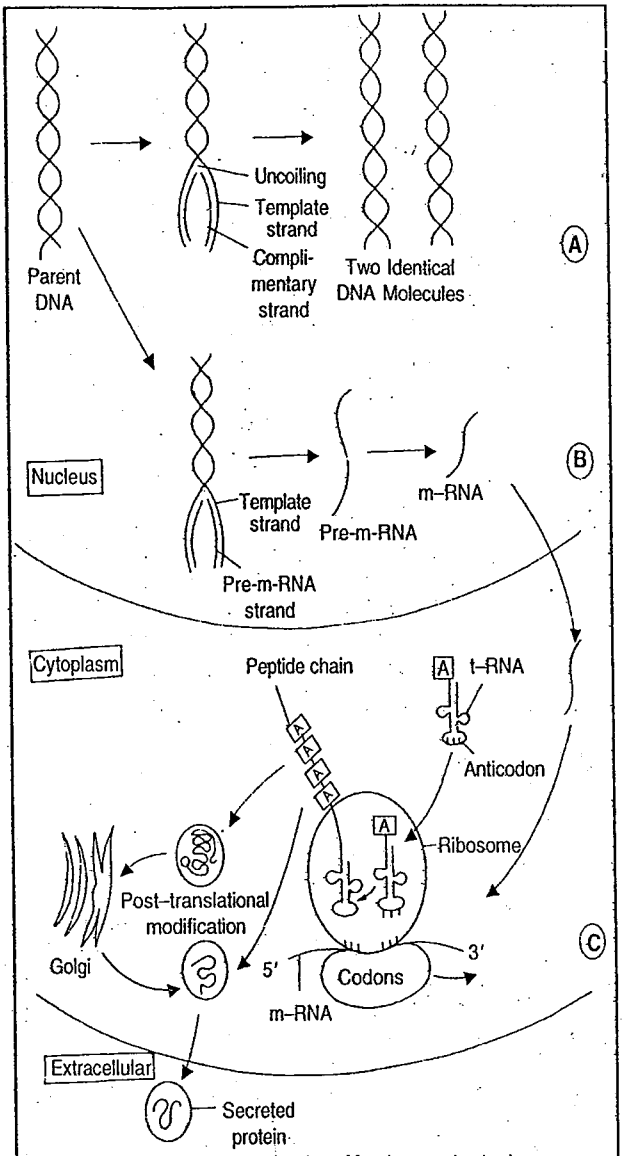


Fig. 9.2. Schematic representation of fundamental genetic processes (see text for detail).
A : Replication, B : Transcription, C : Translation
Abbreviations : DNA = deoxyribonucleic acid ; RNA = ribonucleic acid ; m-RNA = messenger ribonucleic acid ; t-RNA = transfer ribonucleic acid ; A = amino acid.

3. Translation

Translation is the process by which ribosomes convert the information carried by m-RNA to the synthesis of new proteins. Translation requires presence of messenger RNA, ribosomes, transfer RNA (t-RNA), and appropriate protein factors and enzymes in the cytoplasm. The information contained in the m-RNA is in the form of codons, which are a group of three adjacent bases which specify amino acids carried by a t-RNA. Transfer RNA also has a sequence of three nucleotides called an anticodon whose base components pair with the codon found on the m-RNA. When t-RNA binds to the m-RNA codon at the ribosome, a specified amino acid is brought into the assembly line to be transferred to the growing peptide chain (Figure 9.2). The synthesis of proteins from amino acids takes place on the surface of ribosomes. Completed proteins may immediately become functional or may undergo further post-translation modifications in the endoplasmic reticulum or Golgi apparatus.

Molecular Biological Tools

Recombinant biotechnology requires certain molecular biological tools to combine material from an organism with genetic material from a different organism. These include restriction enzymes, a vector, and appropriate host cells.

a. ***Restriction enzymes:*** These are a group of highly specific endonucleases which cut and paste together DNA from different sources. These enzymes first recognise specific sequence in double stranded DNA and cut them. The cut sequence is then inserted or spliced into the gap in the vector cleaved by restriction enzymes (Figure 9.3). The final pasting (ligation) of the inserted DNA sequence and the vector DNA occurs through another enzyme called DNA ligase, which catalyses formation of a phosphodiester bond between adjacent 3'-hydroxyl and 5'-phosphate ends of DNA.

b. ***Vector:*** These are carriers which transmit foreign genetic material into host cells. Most commonly used vectors are plasmids and bacteriophages. Plasmids are autonomously, self-replicating, extra chromosomal circular pieces of DNA which are found in bacteria. A bacteriophage or phage is a virus that infects a bacterial host. Phage lambda is a common bacteriophage that infects *Escherichia coli*. The process of transferring a new gene to a bacterial host via a plasmid or phage vector is termed transformation or transfection, respectively. Many recombinant drugs or reagents are produced with plasmids as vectors.

Host cells: These are cells which receive the recombinant vector and allow its propagation. Selected strains of *E. coli* bacteria are commonly used as host cells for production of recombinant molecules because they are safe, their molecular biology is well characterised, and their single chromosome replicate very fast. Other bacterial cells, yeast and certain mammalian cells are also utilised as host cells. Many recombinant drugs used in the pharmaceutical process are produced with bacterial cells as host; however, products those require post-translation modifications (e.g. recombinant erythropoietin) must be produced in eukaryotic host system like cultured mammalian cells.

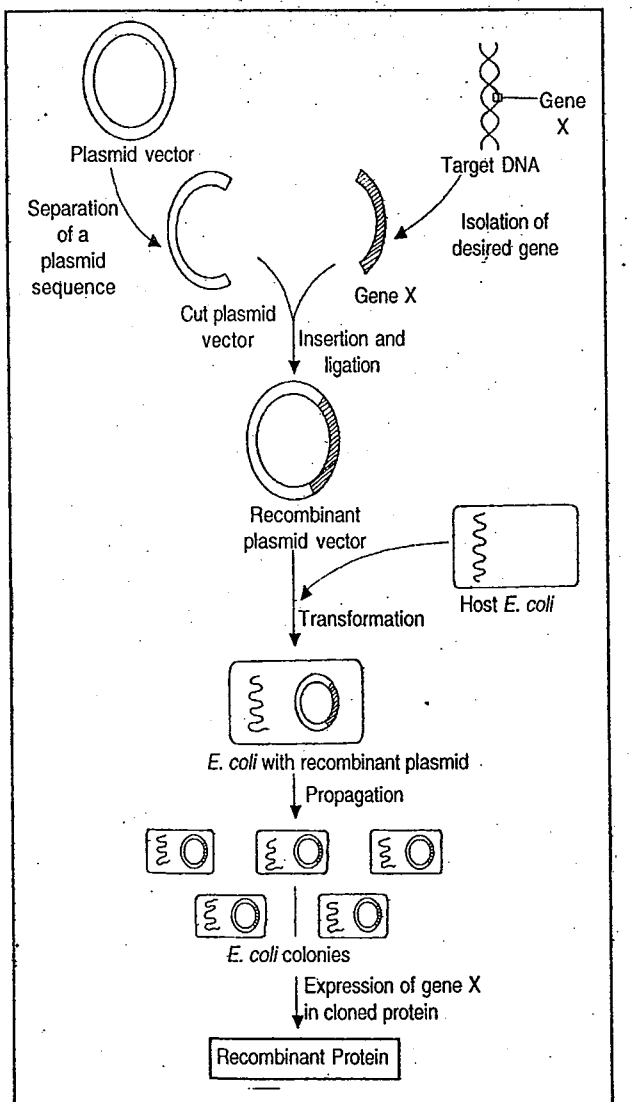


Fig. 9.3. Schematic representation of recominant biotechnology using *E. coli* as host and plasmid as vector (see text for detail).

Procedure

A simplified sequence of production of a hypothetical recombinant product from a desired gene X using *E. coli* plasmids as vectors and *E. coli* cells as hosts is shown in Figure 9.3. Details of the technique, and screening and purification processes are beyond the scope of this text.

In recombinant biotechnology, restriction endonucleases are first employed to cut the required DNA sequence X. After cutting out the specified gene X, the fragment is pasted into a new location in the *E. coli* plasmid by first cutting new site with a restriction enzyme and then inserting the fragment in gap generated. The final connection (ligation) between the inserted gene X and the plasmid DNA is done by action of DNA ligase enzyme. The modified plasmid carrying the new gene is then carefully inserted to the *E .coli* cell. In next step, the host cells transformed with the recombinant plasmid are screened by various techniques to ensure presence of plasmid. When the presence of the recombinant sequence has been verified, large populations of *E .coli* are grown. Finally, the recombinant bacteria synthesised large quantities of proteins are harvested, purified, and produced as a drug. The commercial production of recombinant products requires stringent quality control and extensive testing for purity and quality.

GENE THERAPY

Gene therapy is a genetic engineering technique in which a DNA is used as a drug to supplement or alter defective gene(s) responsible for disease development. The gene therapy was conceptualized in 1972, but was first used successfully in year 1990 in an experiment in the United States, when a patient was treated for ADA-SCID, a congenital disease affecting T cells, resulting from mutation of a gene that codes for adenosine

deaminase leading to severe combined immunodeficiency. Since then, over 1,700 clinical trials have been conducted using a number of techniques for gene therapy.

Types of gene therapy

Gene therapy may be classified into the two important types: somatic gene therapy and germ-line gene therapy. In somatic gene therapy, the therapeutic genes are transferred into the somatic cells or body of a patient. In this type, the modifications in the genes and the therapeutic effects are restricted to the individual patient only and are not inherited to the patient's offspring or later generations. Somatic gene therapy represents the mainstream line of current basic and clinical research, and therapeutic approaches. In germ-line gene therapy, the germ cells i.e. sperm or eggs, are modified by the introduction of functional genes, which are integrated into their genomes. This would allow the therapy to be heritable and passed on to later generations. The germ-line gene therapy is currently not applied clinically for a variety of technical and ethical reasons.

Somatic gene therapy: The somatic gene therapy is currently applied in three categories: *Ex-vivo*, *in situ* and *in vivo*. In *ex-vivo* technique, the cells are removed from the body, incubated with a vector and then gene-engineered cells are returned to body. It is usually done with blood cells because they are easiest to remove and return. The *ex-vivo* gene-therapy has been used in human beings to treat certain diseases like Sickle cell anaemia. *In situ* somatic cell gene therapy, the vector is directly placed into the affected tissues. This type has been used for infusion of adenoviral vectors into the trachea and bronchi of cystic fibrosis patients, and injection of a dystrophin gene directly into the muscle of muscular dystrophy patients in human beings. It has also been used for injection of a tumour mass with a vector carrying the gene for a cytokine or toxin. In the third less commonly practiced *in vivo* gene therapy, the vector is injected directly into the blood stream. There are at present no clinical examples of treating defective gene by *in vivo* technique.

Approaches and Procedures

The gene therapy involves several approaches and procedures. The most common approach of gene therapy involves the insertion of a functional gene at an unspecified location in the host genome to replace a non-functional gene. In other types, an abnormal gene could be swapped for a normal gene through homologous recombination, the abnormal gene could be repaired through selective reverse mutation which returns the gene to its normal function, and the regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

The most common type of gene therapy, where a normal gene is inserted into the host genome to replace an abnormal disease-causing gene, is accomplished by first isolating and copying the gene of interest, generating a construct containing all the genetic elements for correct expression, and then inserting this construct with the help of a carrier molecule, called a vector, to the host cell. Currently, the most common type of vector used in therapeutics is virus which is genetically altered (called viral vector or recombinant virus) to carry normal DNA. Then the target cells are infected with the viral vector, which enters the target cells in a pathogenic manner and then unloads its genetic material containing the therapeutic gene into the cell. Once inside the host cell, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease. A number of viruses have been used for human gene therapy, including retrovirus, adenovirus, lentivirus, herpes simplex virus, vaccinia, pox virus, and adeno-associated virus. Certain other types of vector or methods used to deliver the desired DNA to host cells are the non-viral vectors or non viral-methods. The non-viral methods may include use of liposomes, peptide delivery systems, liposome-polycation complexes, electroporation and inorganic nanoparticles. Non-viral methods present certain advantages over viral methods such as large scale production and low host immunogenicity, however their low levels of transfection and expression of the gene is a disadvantage.

Problems to successful gene therapy

Scientists have been constantly working on gene therapy and have successfully focused on certain human diseases caused by single-gene defects, such as cystic fibrosis, haemophilia, muscular dystrophy and sickle cell anaemia. Gene therapy studies are also aimed at cancer and hereditary diseases linked to a genetic defect. However, there are several problems involved in the successful gene therapy, which may include:

a. Most current gene therapies involve modified viral vectors, which carry risk of causing the disease themselves, if they recover inside the host cell. Viruses also present a variety of potential problems to the patient such as toxicity, immune and inflammatory responses, and gene control and targeting issues.

b. Gene therapies involve carrying large sections of DNA and delivering them to the correct site on the gene. Finding the correct vector to carry such large sections of DNA is a challenge in itself.

c. Most gene therapies available today require multiple dosing of genes due to the short-lived nature of gene therapy. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits.

d. Currently available gene-therapies are effective against diseases caused by mutation in single genes. A large number of diseases such as heart disease, high blood pressure, arthritis and diabetes are caused by multigene or multifactorial disorders and are difficult to treat effectively using currently available techniques.

e. Gene therapy carries risk of immune responses in a patient. The enhanced immune response of the patient to invaders makes it difficult for gene therapy to be repeated in patients.

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CHAPTER 27 AMINE AUTACOIDS

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Autacoids (Greek words: *autos* = self, *akos* = remedy or medicinal agents) are diverse circulating or locally acting hormone like substances, which originate from different tissues and produce intense biological activities near their site of synthesis or release. Autacoids, also called local or tissue hormones, are different from true hormones because they are produced in tissues rather than in glands and are mainly localized in their action. Further autacoids generally act rapidly and briefly, have shorter duration of action, often participate in response to injury, and may have diverse physiological and pathological functions. Some autacoids also serve as transmitters or modulators in the nervous system and may overlap in activity with neurotransmitters and hormones. A number of useful drugs act by modifying synthesis, action or metabolism of autacoids.

Classification

On the basis of the chemical structure, autacoids can be divided into three main groups: amine autacoids, peptide autacoids and lipid derived autacoids.

I. Amine autacoids
   1. Histamine
   2. Serotonin/5-Hydroxytryptamine

II. Peptide autacoids
   1. Kinins (Bradykinin and Kallidin)
   2. Angiotensins
   3. Tachykinins (substance P and neurokinin A)
   4. Cytokines

III. Lipid derived autacoids
   1. Eicosanoids (Prostaglandins, leukotrienes and thromboxane)
   2. Platelet activating factor

AMINE AUTACOIDS

HISTAMINE

Histamine (Greek word: *Histos* = tissue) is a naturally occurring imidazole derivative biogenic amine that is distributed widely in animal tissues and certain plants. In the animal body, histamine plays important roles in regulation of gastric secretion, allergy and anaphylaxis, neurotransmission, haemodynamics, and immune and inflammatory responses. Histamine itself has limited value as a pharmacological agent but its antagonists are widely used in

both human and veterinary medicines to inhibit effects of endogenous histamine.

Chemistry and Distribution

Chemically histamine is 2-(4-imidazolyl) ethylamine, comprising an imidazole ring and an amino group connected by two methylene groups. It is widely distributed in plant and animal kingdoms and is present in many venoms, bacteria and animal foodstuffs. Almost all mammalian tissues contain histamine ranging from less than 1 µg/g to more than 100 µg/g. At the cellular level, histamine is present mostly within storage granules of mast cells and basophils. Tissues having large number of mast cells such as skin, gastrointestinal mucosa, nose, mouth, lungs, liver and placenta hence contain high concentrations of histamine. Non-mast cell histamine occurs mainly in the brain where it may be implicated in the activity of histaminergic neurons. Non-mast histamine also occurs in gastric mucosa, epidermis and growing regions. The non-mast cell histamine is probably of greater physiological importance.

Synthesis, Storage, Release and Metabolism

Synthesis and storage: Histamine is synthesised from the amino acid histidine by action of L-histidine decarboxylase, an enzyme widely distributed in the mammalian tissue. After synthesis, histamine is either stored in tissues or rapidly inactivated. Storage of histamine mainly occurs in mast cells or basophils where it is held in intracellular storage granules in a complex form with an acidic protein and a heparin of high molecular weight. The molar ratio of histamine, heparin and protein in mast cells is 1: 3: 6 and the histamine content is approximately 0.1 – 0.2 pmol per mast cell and 0.01 pmol per basophil. The mast cell histamine in complex form is inactive and cannot be metabolised. The rate of synthesis and turnover in mast cells is slow because once tissues rich in mast cells are depleted of their stores of histamine it may take weeks before concentration of the autacoid returns to normal. Some non-mast cells also contain the histamine, but in most such cells histamine is synthesised and continuously released, but not stored. Accordingly, non-mast cell histamine has more rapid turnover rate than the mast-cell histamine.

Release: Histamine is released from mast cells by a secretory/degranulation process during physiological, mechanical or chemical insult. The release process is initiated by a rise in intracellular Ca^{++} levels, which triggers extrusion of the contents of granules by exocytosis (degranulation). Stimuli for histamine release include interaction of antigen with cell-fixed IgE antibodies or interaction of compliment components C3a and C5a with specific receptors on the cell surface. Destruction of cells as a result of trauma, cold, irradiation, bacterial toxins and venoms may also cause release of histamine from tissues. Agents which increase intracellular cAMP formation (e.g. β-adrenoceptor agonists) inhibit histamine release, whereas some neuropeptides (e.g. substance P) and drugs (e.g. morphine) cause histamine release. Histamine stored in mucosal cells of the stomach can be released by mechanical stimuli such as food and vagal stimulation.

Metabolism and excretion: Histamine is mainly inactivated by histamine-N-methyl transferase to form N-methyl histamine, which can then undergo oxidation by monoamine oxidase (MAO) to form N-methyl imidazole acetic acid (Figure 27.1). Alternately, histamine is metabolised by diamine oxidase (histaminase) to form imidazole acetic acid. In the central nervous system, histamine released into the synapses is primarily broken down by histamine-N-methyltransferase, while in other tissues both enzymes may play a role. Various metabolites of histamine possess little or no biological activity and are excreted in urine. Normally very little histamine is excreted unchanged, but in some neoplastic and other disease conditions such as systemic mastocytosis, gastric carcinoid syndrome or urticaria pigmentosa significant histamine is excreted unchanged. Histamine that is ingested or formed by bacteria in the GI tract is rapidly metabolised in liver and eliminated in urine. Normally, the activity of histamine is terminated within 5 to 15 minutes after its synthesis or release.

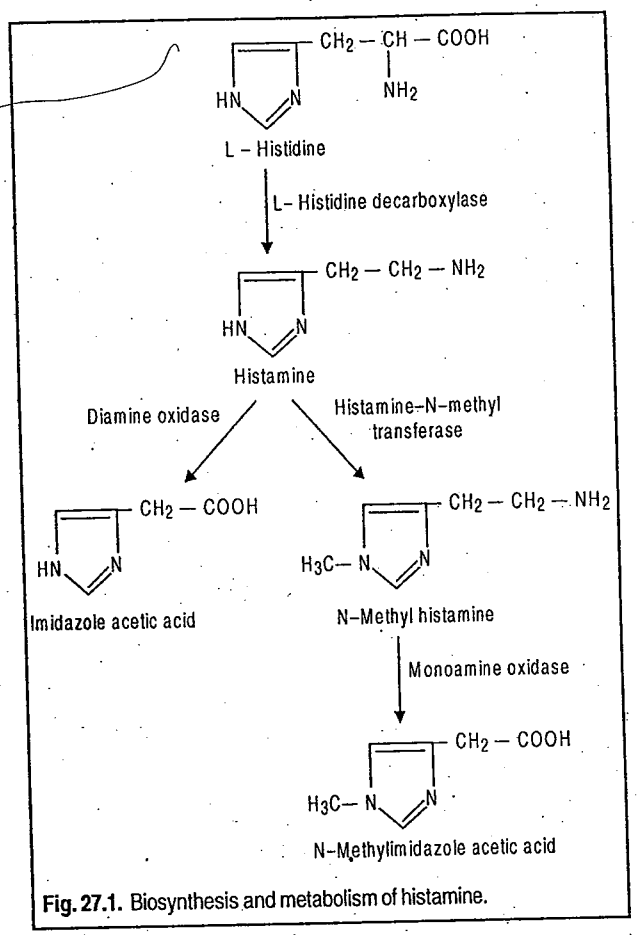


Fig. 27.1. Biosynthesis and metabolism of histamine.

Mechanism of Action

Histamine exerts its actions by combining with specific cellular histamine receptors. Four types of histamine receptors which have been discovered are designated H_1 through H_4, and are all G protein-coupled receptors. The H_1 and H_2 receptors are of clinical importance.

H_1-receptors: The H_1 histamine receptors belong to the superfamily of G-protein-coupled receptors and are expressed throughout the body, especially in smooth muscles, vascular endothelial cells, heart and central nervous system. These receptors are linked to an intracellular G-protein (G_q) that activates phospholipase C and the phosphatidylinositol (PIP_2) signalling pathway. The H_1-receptors are primarily associated with contraction of bronchiolar and intestinal smooth muscle, vasodilatation of small arteries and veins, capillary permeability and pruritus.

In the smooth muscles of bronchi, intestine and large blood vessels, activation of H_1-receptors leads to increased intracellular Ca^{++} concentration through stimulation of phosphatidyl inositol

pathway i.e. H_1-receptor → stimulation of phospholipase C → increased production of inositol-1,4,5-triphosphate → increased release of free calcium from endoplasmic reticulum (see for detail Chapter 6). Enhanced intracellular Ca^{++} in turn activates Ca^{++}/calmodulin dependent myosin light chain kinase. This enzyme phosphorylates the myosin chain with resultant enhancement of cross-bridge cycling and contraction.

In smaller blood vessels, histamine promotes vasodilatation by causing vascular endothelium to release nitric oxide (NO). Nitric oxide diffuses to the smooth muscle cell, where it activates a soluble guanylate cyclase enzyme to cause increased production of cGMP. Stimulation of a cGMP-dependent protein kinase and a decrease in intracellular Ca^{++} are thought to cause vascular relaxation. The H_1-receptor mediated activation of phospholipase A_2 in endothelial cells also leads to formation of prostaglandins, mainly prostacyclin, which may contribute to endothelium mediated vasodilatation in some vascular beds.

2-Methylhistamine is the prototypic H_1-receptor agonist, and mepyramine is the prototypic antagonist. Based on the number of drugs available on market, the H_1-histamine receptor is one of the most clinically important molecular drug target sites.

H_2-receptors: Similar to H_1-receptors, the H_2-receptors are membrane bound G-protein coupled receptors, but are linked to G_s protein. The H_2-receptors are present in gastric glands, blood vessels, uterus and CNS. The H_2-receptors mediate mainly gastric secretion (predominantly H_2 action) and vasodilatation (along with H_1-receptor) and has been associated with inhibition of antibody synthesis, T-cell proliferation and cytokine production. The H_2-receptors activate primarily adenylate cyclase enzyme to form intracellular cAMP. The increased intracellular cAMP activates cAMP-dependent protein kinase, which in turn leads to phosphorylation of a series of enzymes to produce a characteristic response (see also Chapter 6). The H_2-histamine receptors are also associated with the increased intracellular Ca^{++} concentrations and thereby the enhanced release of Ca^{++} from intracellular stores. The mechanism of cAMP-mediated relaxation of smooth muscle, however, is ill understood but it is presumed to involve a decrease in intracellular Ca^{++} levels.

4-Methyl histamine is the prototypic H_2-receptor agonist, and cimetidine is the prototypic antagonist.

H_3-receptors: The H_3-histamine receptors are G-protein coupled receptors coupled to the G_i G-protein. However their functional pathway is not clear, but seems to involve inactivation of adenylate cyclase (↓ cAMP), activation of K^+ channels, or inhibition of Ca^{++} influx. The H_3-receptors are located mainly presynaptically on neurons in brain, where they modulate (inhibit) histamine release. These receptors are also present in lungs, spleen and skin and may be involved in inhibition of histamine release. Their presence in bronchi and ileum has been linked to inhibition of acetylcholine release and in arteries to the vasodilatation. The H_3-receptors have been proposed as a target for treating sleep disorders and for treating neuropathic pain. However, there are currently no therapeutic agents acting as selective agonists for H_3 receptors.

H_4-Receptors: The histamine H_4 receptors, like the other three type of histamine receptors, are members of the G protein-coupled receptor superfamily. The H_4 receptors are highly expressed in bone marrow and white blood cells and regulate neutrophil release from bone marrow. They are also expressed in the colon, liver, lung, small intestine, thymus, spleen, testes,

Table 27.1: Distinctive features of histamine receptor subtypes.

Feature	H_1-Receptor	H_2-Receptor	H_3-Receptor	H_4-Receptor
Distribution	Blood vessels, smooth muscles, CNS, autonomic ganglia, adrenal medulla.	Parietal cells, blood vessels (smaller), heart, CNS.	Brain (presynaptic), lung, spleen, skin, ileum, airways, certain blood vessels.	Bone marrow, and basophils, thymus, small intestine, lungs, spleen, and colon.
Receptor coupling	G-protein coupled (G_q)	G-Protein coupled (G_s)	G-Protein coupled (G_i)	G-Protein coupled (G_i)
Effector pathway	Activation of phoshatidyl cycle i.e. ↑ IP_3/DAG, release of Ca^{++}; ↑cGMP and release of nitric oxide.	Activation of adenylate cyclase i.e. ↑ cAMP; release of Ca^{++}.	Regulation of Ca^{++} influx; activation of K^+ channel; ↓ cAMP.	Inhibition of adenylate cyclase i.e. ↓ cAMP
Biological effects	Vasoconstriction (larger vessels) or vasodilatation (smaller vessels), smooth muscle contraction, neurotransmission, motion sickness and sleep regulation.	Gastric acid secretion, vasodilatation (small vessels), cardiac stimulation.	Decrease neurotransmitter release: histamine (mainly), acetylcholine norepinephrine and serotonin.	Neutrophil release and mast cell chemotaxis.
Selective agonist	2-Methyl histamine 2-Pyridylethylamine	Dimaprit, impromidine	(R) α-Methyl histamine, immethridine, cipralisant.	4-Methylhistamine
Selective antagonist	Mepyramine, chlorphenamine	Cimetidine, ranitidine	Thioperamide, clobenpropit	Thioperamide

Abbreviations: cAMP = 3', 5' cyclic adenosine monophosphate; cGMP = 3', 5' cyclic guanosine monophosphate; DAG = diacyl glycerol; IP_3 = inositol 1,4,5- triphosphate; ↑ = increase; ↓ = decrease.

tonsils and trachea. They have been shown to mediate mast cell chemotaxis. The H_4-receptors mainly act through G_i-coupled decrease in cAMP. 4-Methylhistamine is the prototypic H_4-receptor agonist, and thioperamide is the prototypic antagonist.

Physiological and Pharmacological effects

Main actions of histamine are exerted on the cardiovascular system, smooth muscles and gastric secretions. With a few exceptions, the degree and type of these actions vary from species to species.

Cardiovascular system:

Blood vessels: Histamine causes marked dilation of smaller blood vessels (arterioles, capillaries and venules) in most species resulting in decreased peripheral resistance and fall in systemic blood pressure. Vasodilatation involves both H_1 and H_2 receptors in most vascular beds, but the response differ in their sensitivity to histamine and in duration of effect. The H_1-receptors have high affinity for histamine and mediate an endothelium-NO-dependent vasodilatation that is relatively rapid in onset and short-lived. By contrast, H_2 receptors stimulate the cyclic AMP–PKA pathway in smooth muscle and causes a dilator response that is quick in onset but is short lived. Low concentrations of histamine affects only the H_1 component, whereas large concentrations involve both H_1 and H_2 components; therefore fall in blood pressure due to large doses is completely blocked only by combination of H_1 and H_2 antagonists and not by H_1-antagonists alone. Histamine given in large doses or released during systemic anaphylaxis causes a profound and progressive fall in blood pressure causing shock. In rodents, histamine causes constriction of arterioles, which may overshadow dilation of finer blood vessels. Therefore in rabbits and guinea-pigs, histamine increases total peripheral resistance and causes increase in blood pressure.

In contrast to smaller blood vessels, histamine tends to constrict larger arteries and veins. This effect is mediated by H_1-receptors present on vascular smooth muscle of larger blood vessels.

Capillary permeability: Histamine increases capillary permeability by causing endothelial cells of post-capillary venules to contract and separate at boundaries. The separation of endothelial cells results in outward passage of plasma proteins and fluid into the extracellular space and formation of oedema. This is primarily an H_1-receptor mediated response.

Heart: Histamine produces both positive inotropic and chronotropic effects. It increases the force of contraction of both atrial and ventricular muscles by promoting influx of Ca^{++} and increases the heart rate by hastening diastolic depolarisation in the SA node. These effects are prominent in isolated heart because *in situ* heart baroreceptor reflexes elicited by the hypotension overshadow cardiac responses of histamine. These cardiac responses are mediated mainly by H_2 receptors in heart and also due to fall in blood pressure. Histamine also acts directly to slow atrioventricular (AV) conduction, increase automaticity and in high doses elicit arrhythmias.

Triple response: When skin is scratched vigorously or when histamine is injected intradermally, a characteristic phenomenon known as 'triple response' is produced. It is characterised by a reddening at the localised site, formation of a wheal and a bright crimson flare surrounding the wheal. The reddening at the site results from direct vasodilatory effect of histamine, the wheal reflects histamine's capacity to increase capillary permeability and to cause oedema, and the flare is due to histamine induced stimulation of axon reflexes which cause vasodilatation indirectly. The triple response is accompanied by transient pain and itching caused by histamine-induced stimulation of H_1 receptors on sensory nerve endings.

Smooth muscles: Histamine produces strong contraction of various non-vascular smooth muscles due to activation of H_1 receptors, but may also produce relaxation due to activation of H_2 receptors.

Respiratory system: Activation of H_1 receptors in the bronchial smooth muscle results in bronchoconstriction and decreased lung capacity in most species. Guinea pigs and patients of asthma are highly sensitive. In cats and sheep, activation of H_2 receptors causes bronchodilation.

Gastrointestinal system: In gastrointestinal tract, histamine produces intestinal hypermotility producing spasmodic contractions via H_1 receptors. Large doses cause abdominal cramps and colic by producing intense intestinal contractions.

Uterus: Response of uterus to histamine varies with species. Guinea-pig uterus is contracted while that of rat is relaxed. In human uterus, the response of histamine is negligible.

Other smooth muscles: Urinary bladder, ureter, gall bladder, iris and many other smooth muscles are affected little or inconsistently by histamine.

Exocrine glands: Histamine causes marked increase in gastric secretion, primarily of gastric acid but also of pepsin. This action is exerted on parietal cells of the oxyntic gland through H_2 receptors (see also Chapter 42). Histamine also has some stimulant effect on the lachrymal, salivary, bronchial and pancreatic exocrine glands, but these effects are not very significant.

Other effects: Histamine stimulates the adrenal medulla and causes release of epinephrine. This can cause a secondary rise in blood pressure. It also stimulates the autonomic ganglia. Histamine stimulates various nerve endings and sensory effects. Thus in the epidermis, it causes itch and in the dermis, it evokes pain accompanied by itching.

Patho-physiological Roles

1. ***Allergic reactions and anaphylactic shock:*** Histamine is one of several autacoids which participate in hypersensitivity reactions. Antigenic substances cause the release of histamine when they bind to immunoglobulin E (IgE) molecules located on the mast cell membrane. Therefore, histamine is suggested to be causative in urticaria, bronchoconstriction and anaphylactic shock.
2. ***Inflammation:*** Histamine has been implicated as a mediator of vasodilatation and other changes which occur during inflammation. It promotes adhesion of leucocytes to vascular endothelium by expressing adhesion molecule P-selectin on endothelial cell surface and sequestering leucocytes at the inflammatory sites.
3. ***Gastric secretion:*** Histamine is present in high concentration in the gastric mucosa and is a physiological mediator of gastric secretion (see for detail, Chapter 42).
4. ***Neurotransmission:*** Histamine is believed to be involved in the initiation of sensory impulses of pain and itching. Non-mast cell histamine occurs in brain, especially hypothalamus and mid-brain, and appears to be involved

in maintaining wakefulness and regulating temperature, cardiovascular function, and thirst (see also Chapter 17).

5. ***Tissue repair and growth:*** A histamine-synthesising capacity is found in rapidly proliferating tissues such as liver and bone marrow. Therefore, histamine has been suggested to play an essential role in the process of growth and repair.
6. ***Regulation of microcirculation:*** Histamine plays a role in regulation of microcirculation through its vasoactive properties.
7. ***Malignancies and tumours:*** Increased number of mast cells or basophils is associated with many tumours like urticariapigmentosa, myelogenous leukaemia and gastric carcinoid tumours with increased excretion of histamine and its metabolites.

PHARMACOLOGY OF HISTAMINE AND ANALOGUES

Histamine

Histamine has no therapeutic use because of its profound adverse effects. However, it is infrequently used as a diagnostic tool to test acid secreting capacity of stomach and to distinguish between pernicious anaemia and other forms of anaemia. The loss of gastric parietal cells in pernicious anaemia results in an inability to secrete gastric acid even in response to histamine, but in other types of anaemia, gastric acid secretion increases in response to histamine. As histamine is not effective orally due to its rapid inactivation in the GI tract and liver hence it is administered by parenteral route. Absorption of histamine is virtually complete after parenteral injection, but its duration of action is brief because of rapid metabolism and distribution into tissues. Exogenous histamine is also incorporated into storage granules to some extent, but this pathway is not important. Important side effects of exogenous histamine injection include hypotension, tachycardia, bronchoconstriction, dyspnoea, diarrhoea and rise in body temperature. In such conditions, the side effects of parenteral histamine is minimised by pre-treatment with an H_1 receptor antagonist (e.g. mepyramine), which antagonises H_1 responses but does not interfere with the acid secretion that is a H_2 receptor response.

Histamine is commercially available as histamine phosphate. Doses of histamine are expressed conventionally in terms of base, and 2.75 mg of histamine phosphate is equivalent to 12 mg of histamine base.

Dose

Histamine acid phosphate

Dogs : 0.04 mg/kg, SC.

Betazole

Betazole (Ametazole) is a histamine H_2 receptor agonist and an isomer of histamine with preferential effects on gastric secretion. It is about 10 times more potent as a stimulator of gastric secretion than a vasodilator. It may be used as an alternative for histamine phosphate in tests of gastric function because of its specificity for the H_2 receptor and its advantage of not generating the undesirable side effects which histamine would induce. It therefore does not require concomitant use of a H_1 receptor antagonist to block the actions of histamine at other histamine receptor types in body.

Dose

Dogs : 1.7 mg/kg, SC.

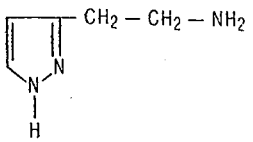


Fig. 27.2. Chemical structure of betazole.

Pentagastrin

Pentagastrin (N-(isobutoxycarbonyl)-β-alanyl-L-tryptophyl-L-methionyl-L-α-aspartyl-L-phenylalaninamide) is a synthetic pentapeptide having secretory responses similar to those of histamine and betazole. It stimulates the secretion of gastric acid, pepsin and intrinsic factor, and has been used as a diagnostic aid. It is more commonly used for gastric analysis because it is short acting and has minimal side effects. Pentagastrin is also used as a stimulation test to elevate serotonin levels and cause symptoms of carcinoid syndrome. The pharmacology of pentagastrin is given in Chapter 42.

Betahistine

Betahistine is chemically 2-[2-(methylamino)ethyl]pyridine and it structurally resembles histamine. It has been used in human medicine to control vertigo in patients of Meniere's disease. Betahistine has a weak affinity as an agonist for histamine H_1 receptors, but a very strong affinity as an antagonist for histamine H_3 receptors. Betahistine seems to dilate the blood vessels within the middle ear which can relieve pressure from excess fluid and act on the smooth muscle.

HISTAMINE BLOCKING DRUGS

The effects of histamine in the body can be blocked in three different ways:

1. ***Prevention or reduction of histamine release from sensitised mast cells:*** Some drugs stabilise the mast cell membrane and/or suppress the tissue effects of antigen-antibody reactions. These drugs, therefore, act by suppressing release of histamine from sensitised mast cells. Important drugs of this group include sodium cromoglicate (Chapter 46), corticosteroids (Chapter 51) and newer non-sedating H_1 receptor antagonists (e.g. cetirizine)
2. ***Use of physiological antagonists:*** Some drugs (e.g. epinephrine) oppose actions of histamine on smooth muscle, arterioles and vascular permeability. These drugs are useful to antagonise biological actions of endogenous histamine, particularly in conditions like hypersensitivity reactions. These drugs, however, have no direct action on release or action of histamine and are not usually considered as histamine blocking drugs.
3. ***Histamine receptor antagonists:*** Certain drugs prevent actions of histamine in body by competitively blocking the histamine receptors (H_1 or H_2). These drugs are widely used in therapeutics and are commonly referred to as anti-histamines or histamine receptor blocking drugs. This group of drugs will be discussed in the present section.

HISTAMINE RECEPTOR ANTAGONISTS

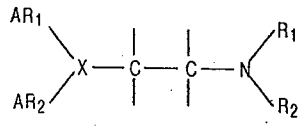
Histamine receptor antagonists or antihistamines are drugs, which competitively block action of histamine at the histamine receptor sites. Depending on their affinity for histamine receptor subtypes, these drugs are broadly classified into two major groups: H_1-receptor antagonists and H_2-receptor antagonists. Although agents which act selectively at H_3-receptors and H_4-receptors have also been developed recently, but such drugs are not yet available for clinical use.

A. H_1-RECEPTOR ANTAGONISTS

The H_1-Receptor antagonists comprise a large group of agents which selectively inhibit H_1-receptors. These agents are sometimes referred to as classic or conventional antihistamines (antihistaminics) because they were the first to be discovered. These agents are widely used in the treatment of allergic disorders, prevention of motion sickness and as an adjunct in systemic anaphylaxis.

Chemistry and structure activity relationship

Most H_1-receptor antagonists are substituted ethylamines i.e. they contain a substituted ethylamine moiety ($-CH_2-CH_2-N<$). This ethylamine moiety is also present in histamine; therefore, it is considered to be the molecular component required for drug-receptor interaction. However unlike histamine that has a primary amino group and a single aromatic ring, most H_1 antagonists contain a tertiary amino group linked via a two- or three-atom chain to two aromatic substituents. The chemical structure of most H_1-receptor antagonists can be depicted by the general formula shown below.



Basic structure

Fig. 27.3. Basic structure of H_1-receptor antagonists.

In this formula, AR is an aryl group, X is a nitrogen, carbon or oxygen, and R is an alkyl group. Sometimes the two aromatic groups (AR_1 and AR_2) are bridged (e.g. phenothiazines) or the ethylamine may be part of a ring structure (e.g. piperazines). In general, maximum antihistaminic effect is achieved, when H_1 receptor antagonist structure consists of two carbon chain and dimethyl nitrogen atom. Other variations also are possible; for example, terfenadine and astemizole have aromatic ring structures on each side of carbon chain.

Classification

H_1-receptor antagonists are broadly categorised on the basis of their origin into two groups – first generation antihistaminics and second generation antihistaminics. Each group is further subdivided according to chemical structures of the drugs.

I. First-generation H_1 receptor antagonists/Sedating antihistamines
   1. Ethanolamines (X = C-O)
      e.g. diphenhydramine, dimenhydrinate, clemastine, doxylamine, carbinoxamine and embramine.
   2. Ethylenediamines (X = N)
      e.g. mepyramine, tripelennamine, antazoline and methapyrilene.
   3. Alkylamines (X = C)
      e.g. chlorphenamine, pheniramine, dexchlorphenamine, brompheniramine, triprolidine and dimethindene.
   4. Piperazines (X = C-N)
      e.g. hydroxyzine, meclozine, cyclizine, chlorcyclizine, buclizine and cinnarizin.
   5. Phenothiazines (X= N)
      e.g. promethazine, alimemazine, dimethothiazine and methdilazine.
   6. Miscellaneous
      e.g. cyproheptadine, phenindamine, azatidine and mebhydrolin.

II. Second generation H_1 -receptor antagonists/Non-sedating antihistamines
   1. Piperazines
      e.g. cetirizine and levocitirizine.
   2. Piperidines
      e.g. terfenadine, astemizole, fexofenadine, loratadine, desloratadine, mizolastine and ebastine.
   3. Miscellaneous
      e.g. azelastine, mequitazine, acrivastine, rupatadine and olopatadine.

Mechanism of action

The H_1 receptor antagonists do not influence formation or release of histamine, but they antagonise actions of histamine at H_1-receptor sites. These drugs block all actions of histamine except for those mediated solely by H_2-receptors. In recent years, it has been shown that these drugs are actually H_1-receptor inverse agonists rather than H_1-receptor antagonists as they stabilise the H_1-receptors in inactive state. However, the older concept still prevails and even today they are commonly called H_1-receptor antagonists. In addition to H_1 blocking action, many of these drugs tend to block muscarinic receptors and a few drugs also antagonise adrenergic or serotonin receptors.

I. First-Generation H_1-Receptor Antagonists

1. Ethanolamines

Ethanolamines are organic chemical compounds which possess both a primary amine (due to an amino group in its molecule) and a primary alcohol (due to a hydroxyl group). Several ethanolamines are used in therapeutics, some as histamine H_1- receptor blockers.

Diphenhydramine

Diphenhydramine HCl (DPH) is an ethanolamine-derivative H_1-receptor antagonist and the prototypic drug of its class. It was one of the first known antihistamines, invented in 1943 by George Rieveschl. In addition to H_1 receptor blocking action, diphenhydramine possesses significant sedative and anti-muscarinic activities. It also has substantial local anaesthetic, anti-tussive and antiemetic properties.

Pharmacological effects

The diphenhydramine and other H_1-receptor antagonists do not produce cellular actions of their own, but they competitively block action of histamine at H_1-receptors in the tissue cells. Therefore, their effects are largely predictable from knowledge of the responses to histamine which involve interaction with H_1 receptors. In general,

they are more effective against exogenously administered histamine than against endogenously released histamine, and in preventing actions of histamine than in reversing them.

Anti-histaminic effects:

Cardiovascular system: Diphenhydramine and other H_1-receptor antagonists inhibit most responses of cardiovascular system to histamine. Diphenhydramine inhibits histamine-induced vasodilatation that is mediated by H_1 receptors on smaller blood vessels. Fall in blood pressure produced by low doses of histamine is blocked but additional H_2-antagonists are required for complete blockade of high doses. It strongly blocks the action of histamine that results in increased capillary permeability and formation of oedema, and suppresses triple response. The pharmacological effects of diphenhydramine include reduction in swelling, hyperthermia and redness at site of inflammation.

Smooth muscles: Diphenhydramine inhibits most responses of non-vascular smooth muscle to histamine. These drugs effectively block histamine-induced contraction of bronchi, intestine and other smooth muscles.

Anti-allergic effects: Diphenhydramine and other H_1-blockers counteract urticaria, whealing and other types of oedema formation in response to allergens, antigens, injury or histamine liberating drugs. They inhibit pruritus, itching and pain sensations associated with allergic reactions by preventing stimulations of sensory nerves. The protection afforded by diphenhydramine against hypersensitivity reactions varies widely with species and tissues because histamine is one of many potent autacoids released during such reactions against which antihistamines are ineffective.

Exocrine glands: Diphenhydramine and other H_1-antagonists do not inhibit gastric secretion but they suppress respiratory, salivary, lachrymal and other exocrine glands secretions with variable responses. The effect on exocrine glands is largely attributed to antihistaminic and antimuscarinic effects of drugs.

Adrenal medulla: Diphenhydramine abolishes the histamine-evoked release of catecholamines from adrenal medulla.

Central nervous system: Diphenhydramine produces significant degree of CNS depression and sedation. In some individuals or in very high doses, there may be CNS stimulation characterised by restlessness, nervousness and rarely convulsions.

Other effects: Diphenhydramine has additional effects unrelated to its binding of H_1 receptors. These effects probably reflect its binding to other sites such as cholinergic receptors and sodium channel.

Anti-cholinergic effects: Diphenhydramine and some other first generation H_1 antagonists tend to inhibit responses to acetylcholine that are mediated by muscarinic receptors. This action produces atropine-like effects such as xerostomia, dysuria, blurring of vision and decreased GI motility. The central antimuscarinic actions of diphenhydramine are considered useful in the treatment of motion sickness. Inhibition of histamine effects at vomiting centre and vestibular centres are also involved.

Local anaesthetic effect: Diphenhydramine acts as a sodium channel blocker, which is responsible for its membrane stabilising action and local anaesthetic effect. The local anaesthetic effect may contribute to the relief of pruritus and itching on topical application.

Antitussive effect: Diphenhydramine possesses significant antitussive effect.

Pharmacokinetics

Diphenhydramine is a lipid soluble amine hence is readily absorbed on oral or parenteral administration. After oral absorption, it undergoes a relatively high first-pass effect, so that only about 40-60% of drug reaches the systemic circulation. It is widely distributed into body tissues and enters brain and placenta. The onset of action is usually within 30 minutes following oral administration and duration is between 4 to 6 hours. It is primarily metabolized in liver and majority of drug is excreted as metabolites in urine.

Side/Adverse effects

The incidence and severity of side effects are related to the peripheral and central H_1-receptor and muscarinic-cholinergic receptor blockade. Important side effects of diphenhydramine include sedation, diminished alertness, lethargy, somnolence and ataxia due to CNS depression and dry mouth, dysuria, constipation, increased heart rate and pupil dilation due to anticholinergic effects. These side effects may adversely affect the performance of working animals. Diphenhydramine may also produce gastrointestinal disorders like nausea, vomiting or gastric distress in simple stomach animals, especially when oral administration is prolonged or when drug is administered empty stomach. Higher concentrations of diphenhydramine may produce CNS stimulation and excitement, or severe CNS depression and respiratory arrest, especially after IV administration or toxic doses.

Treatment: Diphenhydramine overdosage may be treated symptomatically and with decontamination therapy. Phenytoin (IV) is recommended in the treatment of seizures caused by diphenhydramine and other antihistamines overdosage.

Contraindications and precautions

Diphenhydramine and other H_1 receptor antagonists should not be used in patients those are hypersensitive to antihistamines. Because of their anticholinergic properties, H_1 antagonists should be used cautiously in patients with angle-closure glaucoma, prostrate hypertrophy, pyloroduodenal or bladder neck obstruction, and chronic obstructive pulmonary disease (COPD) (if mucosal secretion is a problem). They should be used with caution in patients with hyperthyroidism, cardiovascular disease or hypertension.

Drug interactions

CNS depressant drugs can potentiate the sedative effect of diphenhydramine and other H_1 antagonists. Diphenhydramine and some other antihistamines may antagonise effects of antihypertensive drugs and may enhance effect of drugs like epinephrine. The H_1 antagonists may partially counteract the anticoagulant effects of heparin or warfarin.

Clinical uses

In veterinary medicine, diphenhydramine is used mainly for the control and prevention of various allergic disorders like urticaria, pruritus, angioedema and systemic anaphylaxis. For anaphylaxis or severe urticaria and angioedema, diphenhydramine is often used in conjunction with corticosteroids and epinephrine. Its sedative and local anaesthetic effects are particularly useful in treating the agitation associated with allergic responses. Diphenhydramine is also used clinically for the treatment and prevention of motion sickness and as antiemetic in small animals (see also Antiemetics, Chapter 44). Its antimuscarinic properties contribute to its ability to relieve upper respiratory tract irritation

during cough. Diphenhydramine has a powerful hypnotic effect and for this reason is often used as a non-prescription sleep aid and a mild anxiolytic. In human medicine, it may also be used for the treatment of extrapyramidal side effects of many antipsychotics

Dose

For allergic disorders

Dogs & cats : 2 – 4 mg/kg, PO, 3 to 4 times daily.
: 2 mg/kg, IM or slow IV, 2 times daily or as needed.

For adjunctive therapy of anaphylaxis

Cattle : 0.5 – 1 mg/kg, IM or IV.
Horses : 0.25 – 1 mg/kg, IV or IM.

Note: The IV route should not be used routinely because it carries risk of cardiac arrest.

Clemastine

Clemastine (Meclastin) fumarate is an ethanolamine antihistamine with significant sedative and antimuscarinic effects. Similar to diphenhydramine, it blocks action of histamine on H_1-receptors and reduces inflammatory and allergic signs such as increased capillary permeability, oedema, swelling, pruritus and itching. It also decreases gastrointestinal and respiratory tract smooth muscle constriction. Clemastine appears to be very effective in stopping itching associated with pruritus in dogs and cats. It is also indicated in human practice for use in treating allergic rhinitis, such as sneezing, rhinorrhoea and lacrimation. The half-life of clemastine in dogs is about 3.5-4 hours and it has rapid clearance. Side/adverse effects are similar to diphenhydramine and include mainly sedation, lethargy, dry mouth, constipation at normal dosage and CNS depression or stimulation at higher dosages.

Dose

Dogs : 0.05 – 0.1 mg/kg, PO, 2 times daily.
Cats : 0.67 mg (total), PO, 2 times daily.
Horses : 0.05 mg/kg, IV, 3 times daily.

Other ethanolamines

Dimenhydrinate, carbinoxamine, doxylamine and embramine are other ethanolamine-derivative antihistamines. Like diphenhydramine, these drugs possess significant antimuscarinic activity and have a pronounced tendency to induce sedation. Dimenhydrinate is occasionally used in veterinary medicine as an antiemetic for prevention and control of motion sickness (see for detail Antiemetics, Chapter 45) and also as a cough suppressant. Carbinoxamine is primarily used for treatment of vasomotor rhinitis, mild urticaria, angioedema and allergic conjunctivitis. Doxylamine is used by itself as a short-term sedative and in combination with other drugs as anti-allergic drug. Embramine is used in human medicine as an appetite depressant and for the treatment of persons suffering from narcolepsy and attention deficit hyperactivity disorder (ADHD).

Ethylenediamines

Mepyramine

Mepyramine (Pyrilamine) maleate is an ethylenediamine H_1-receptor antagonist. It is highly specific for H_1 receptors (relative selectivity for H_1 : H_2 receptors is 6000: 1) and has less sedative and much less anticholinergic effects when compared with diphenhydramine. The pharmacokinetics of this drug has apparently not been extensively studied in veterinary species, but is reported to have a short duration of action. In veterinary medicine, mepyramine is used occasionally to reduce or help prevent histamine mediated adverse effects. It is also used topically as cream for the treatment of insect bites and stings nettle rash. Adverse effects of systemic mepyramine in horses include CNS stimulation (nervousness, ataxia, tremors and convulsions) and GI disturbances (anorexia and gastric distress). Alternatively, CNS depression with sedation may be observed.

Dose

Dogs : 12.5 – 25 mg (total), PO, 4 times daily.
Horses : 1 mg/kg, slow IV, IM or SC.
Cattle : 0.5 – 1.5 g (total), IM.

Tripelennamine

Tripelennamine HCl is an ethylenediamine derivative antihistamine. It possesses significant H_1 receptor blocking activity, but has moderate sedative and minimal antimuscarinic activities. In addition to its antihistamine and anticholinergic effects, tripelennamine also functions as a weak serotonin reuptake inhibitor (SRI) and dopamine reuptake inhibitor (DRI). Tripelennamine has short to medium duration of action and is used in veterinary medicine to reduce or prevent histamine mediated adverse effects. It has also been used as a CNS stimulant in 'Downer cows' when administered by slow IV route. In human medicine, tripelennamine is also used occasionally as a psychoactive drug. Important side effects of tripelennamine in different species include CNS depression, incoordination and GI disturbances. Tripelennamine is not recommended by IV route in horses because it causes excessive CNS stimulation.

Dose

For histamine induced adverse effects

Dogs & cats : 1 mg/kg, PO or IM, 2 times daily.
Horses & cattle: 1 mg/kg, IM, 2 to 3 times daily or as needed.
Swine : 1 mg/kg, IM or IV, 2 to 4 times daily.

For adjunctive therapy of 'Downer Cow syndrome'

Cattle : 0.5 mg/kg, slow IV in conjunction with parenteral mineral treatment.

Other ethylenediamines

Some other ethylenediamines possessing H_1 antagonistic properties include antazoline and methapyrilene. Antazoline hydrochloride is less potent but also less locally irritant than many antihistamines. It is, therefore, used mainly to relieve nasal congestion and in eye drops, usually in combination with naphazoline, to relieve the symptoms of allergic conjunctivitis. Methapyrilene has relatively strong sedative effects to the extent that its primary use is a hypnotic rather than as an antihistaminic drug. Methapyrilene is less used in therapeutics due to its potential to cause liver damage.

3. Alkylamines

Chlorphenamine

Chlorphenamine (Chlorpheniramine) maleate is an alkylamine H_1 receptor antagonist. Chlorphenamine is among the most potent and most selective H_1 antagonists (relative selectivity for H_1: H_2 receptors is 15000: 1). In addition to being histamine H_1 receptor antagonist, chlorphenamine has been shown to work as a serotonin-norepinephrine reuptake inhibitor (SNRI). It has less sedative effect than diphenhydramine and is not likely to produce intense

drowsiness. Chlorphenamine maleate is well absorbed after oral administration, but undergoes some degree of first pass metabolism in liver. It is well distributed in body, metabolised mainly in liver and excreted both as metabolites and unchanged drug in urine.

Chlorphenamine maleate is one of the most commonly used antihistamines in small-animal veterinary practice. It is commonly used as premedication for drugs which may induce an anaphylactic reaction (e.g. cytotoxic drugs), to control pruritus in allergic skin disorders, and in behaviour modification programmes. Although not generally approved as an antidepressant or anti-anxiety medication, chlorphenamine appears to have these properties as well. Chlorphenamine is often combined with phenylpropanolamine to form an allergy medication with both antihistamine and decongestant properties. It is also combined with the cough suppressant dextromethorphan to produce antiallergic and antitussive effects. Important adverse effects of chlorphenamine include CNS depression and GI disturbances.

Dose

For pruritus in allergic skin disorders

Dogs : 4 – 8 mg (total), PO, 2 to 3 times daily.

Cats : 2 – 4 mg (total), PO, 2 times daily.

Premedication

Dogs & cats : 5 – 10 mg (total) diluted in syringe with blood, slow IV over 1 minute.

Behaviour modification

Dogs : 0.2 mg/kg, PO, 3 times daily.

Cats : 1 – 2 mg (total), PO, 2 to 3 times daily.

Pheniramine

Pheniramine (Avil) is an alkylamine antihistamine with anticholinergic properties. It structurally and pharmacologically resembles chlorphenamine. However, it possesses significant CNS depressant and sedative effect in a manner similar to diphenhydramine. Pheniramine is commonly used to treat allergic conditions such as hay fever or urticaria. Similar to chlorphenamine, it is commonly used as premedication for drugs which may induce an anaphylactic reaction (e.g. cytotoxic drugs). Due to its relatively strong sedative effects, pheniramine may sometimes be used off-label as a hypnotic to induce sleep. Important side effects of pheniramine may include sedation, lethargy and drowsiness. Overdose may lead to CNS excitement and seizures.

Other alkylamines

Dexchlorphenamine, brompheniramine, triprolidine and dimethindene are some other alkylamine H_1-receptor antagonists available for therapeutic purposes. Dexchlorphenamine, the pharmacologically active dextrorotatory isomer of chlorphenamine and an antihistamine with anticholinergic properties, is used in veterinary practice to treat allergic conditions. However, it seems to possess no advantage over the more commonly used parent drug. Brompheniramine, the halogenated derivatives of pheniramine, and triprolidine (dipheniramine) are mainly indicated in human medicine for the treatment of the symptoms of the common cold and allergic rhinitis, such as rhinorrhoea, itchy eyes, watery eyes and sneezing. Dimethindene is used in hypersensitivity reactions, in rhinitis, for pruritus, and in some common cold remedies.

4. Piperazines

Hydroxyzine

Hydroxyzine hydrochloride or pamoate is a first-generation antihistamine of the piperazine class. It was first synthesized in 1956 and is still in widespread use today. Its predominant mechanism of action is as a potent H_1 receptor antagonist/inverse agonist. However, unlike most other first-generation antihistamines, it has low affinity for the muscarinic cholinergic receptors and therefore does not produce much clinically significant anticholinergic effect. In addition to its antihistamine activity, hydroxyzine has been shown to act as a serotonin 5-HT_{2A}, dopamine D_2 and α_1-adrenergic receptor antagonist. It also has significant sedative, tranquillising, antispasmodic, local anaesthetic and antiemetic properties. Hydroxyzine is rapidly absorbed after oral and intramuscular administration. It is widely distributed in body and metabolised in liver; the main metabolite is cetirizine. Hydroxyzine is a moderate to long-acting compound with duration of 6 to 8 hours in dogs and up to 12 hours in cats.

Hydroxyzine is used primarily as an antihistamine for the treatment of itching, allergies, hyperalgesia, motion sickness-induced nausea and insomnia. It is also indicated for the treatment of mild anxiety. In veterinary medicine, hydroxyzine is used mainly for its antihistaminic, antipruritic and sedative/tranquillising properties, often in atopic patients. Its considerable central depressant activity may contribute to its prominent antipruritic action. Its other uses include allergic airway disease and rhinitis. Important side/adverse effects associated with hydroxyzine are sedation and gastric disturbances. Occasionally in dogs, it produces hyperexcitability (tremors and rarely seizures).

Dose

For pruritus

Dogs : 1 – 2 mg/kg, PO or IM, 3 to 4 times daily.

Horses : 0.5 – 1 mg/kg, PO or IM, 2 times daily.

Meclozine

Meclozine (Meclizine) hydrochloride is a first-generation antihistamine of the piperazine class. Meclozine is structurally and pharmacologically similar to hydroxyzine. It is primarily an antagonist at H_1-receptors, but also possesses anticholinergic, central nervous system depressant and local anaesthetic effects. It possesses good antiemetic and antivertigo effects which are likely due to combination of its central anticholinergic properties and suppression of labyrinth excitability and vestibular stimulation. It may also directly affect the chemoreceptor trigger zone in the medulla oblongata. Meclozine is used as an antivertigo/antiemetic agent, specifically in the prevention and treatment of nausea, vomiting and dizziness associated with motion sickness. The pharmacology of meclozine is given in Chapter 44.

Other piperazines

Cyclizine, chlorcyclizine, buclizine and cinnarizine are some other piperazine antihistamines. Similar to meclozine, cyclizine is principally used as an antiemetic and for treatment and prevention of motion sickness in small animals (see for detail Antiemetics, Chapter 44). Chlorcyclizine is used primarily to treat allergy symptoms such as rhinitis, urticaria and pruritus, and may also be used as an antiemetic. Buclizine and cinnarizine are considered to be antiemetic, similar to meclozine.

5. Phenothiazines

Promethazine

Promethazine hydrochloride is a phenothiazine-derivative antihistamine. In addition to H_1 receptor antagonistic property, it possesses considerable anticholinergic, sedative, anti-motion sickness and antiemetic actions. It is also a weak dopamine receptor antagonist. In veterinary medicine, promethazine is used primarily against allergic disorders. Its other uses may include as a sedative, hypnotic, antiemetic, antitussive and rarely an antipsychotic. Central nervous system depression with sedation and drowsiness are important side effects.

Dose

For allergic disorders

Dogs : 0.2 – 1 mg/kg, PO, IM or SC, 2 to 3 times daily.

Alimemazine

Alimemazine (Previously trimeprazine) tartrate is a phenothiazine-derivative H_1 receptor antagonist that is related to promethazine. It has antihistaminic, sedative, antitussive and anti-pruritic properties. It is used mainly for the treatment of pruritic conditions, especially those induced by allergic conditions and eczema. It is also occasionally used as a sedative, hypnotic and anti-emetic for prevention of motion sickness.

Dose

For allergic disorders

Dogs : 1 – 2 mg/kg, PO, 3 times daily.

For vomiting (as antiemetic)

Dogs : 0.2 – 0.4 mg/kg, IM or PO, every 4 hours or as needed.

Other phenothiazines

Some other phenothiazine H_1-receptor antagonists include dimethothiazine and methdilazine. These antihistaminic drugs are less commonly used now.

6. Miscellaneous Drugs

Cyproheptadine

Cyproheptadine hydrochloride is an antihistaminic and antiserotonergic agent. It also acts as a muscarinic receptor antagonist as well as a calcium channel blocker. In veterinary practice, cyproheptadine may be used as an antihistamine in treatment of allergic conditions in small animals and for prophylaxis

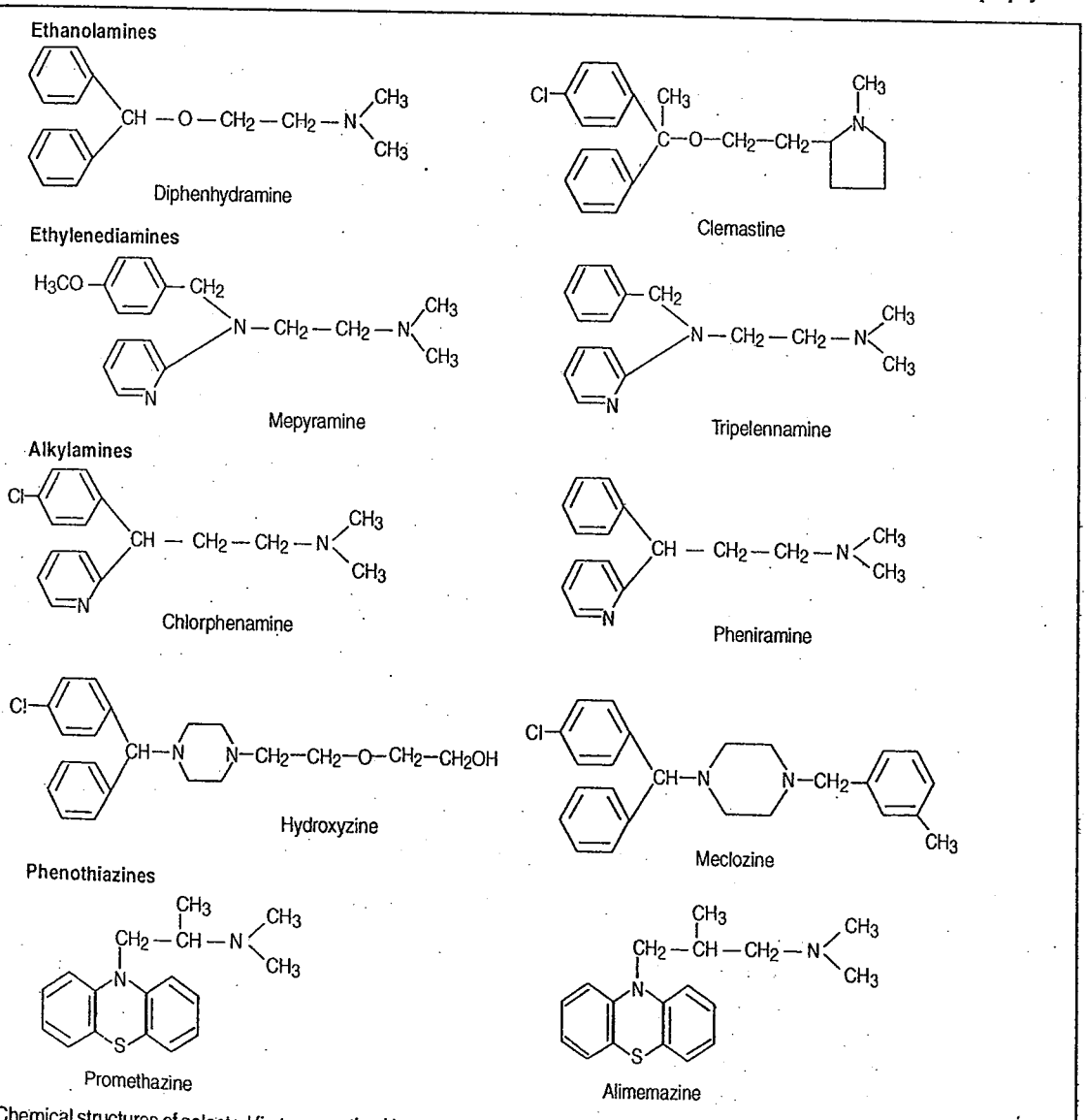


Fig. 27.4. Chemical structures of selected first-generation H_1-receptor antagonists.

in feline asthma. In addition to these, cyproheptadine has other uses in animals such as an appetite stimulant in cats and as adjunctive therapy for pituitary dependent hyperadrenocorticism. In human medicine, cyproheptadine has been used in various conditions including in the management of moderate to severe cases of serotonin syndrome (a complex of symptoms associated with the use of serotonergic drugs), and in the carcinoid disease in which serotonin is overproduced by tumour cells. Important side effects related to cyproheptadine in animals include CNS depression (sedation) and anticholinergic (xerostomia and urine retention) effects. At high doses, it may cause significant polyphagia in dogs. The brief pharmacology and uses of cyproheptadine have also been listed in other relevant sections (see also Chapters 44, 46, 49 and 51).

Dose

For histamine induced adverse effects

Dogs : 1 mg/kg, PO, 2 to 3 times daily.

For feline asthma

Cats : 0.2 – 0.5 mg/kg, 3 times daily.

Other drugs

Phenindamine, azatidine and mebhydrolin are some other drugs possessing significant H_1-receptor antagonistic action. Phenindamine and azatidine are closely related to cyproheptadine and are used to treat symptoms of the allergies and common cold, such as sneezing, itching, rashes and hives. Mebhydrolin (Mebhydroline) is primarily used for symptomatic relief of allergic symptoms caused by histamine release, including nasal allergies and allergic dermatosis.

II. Second Generation H_1-Receptor Antagonists

The second-generation H_1 receptor antagonists (non-sedating antihistamines) are newer H_1-receptor blocking drugs which do not cross the blood-brain barrier and hence have no CNS depressing effect. They also have no anticholinergic or anti-serotonergic activities. Second generation H_1-receptor antagonists are relatively less used in veterinary practice because these drugs apparently have not been studied for efficacy or safety in animals.

1. Piperazines

Cetirizine

Cetirizine is an active metabolite of hydroxyzine that is now available as a peripheral H_1-receptor antagonist drug. Commercially available cetirizine contains l- and d-stereoisomers. Similar to hydroxyzine, cetirizine acts by blocking the histamine H_1 receptors. However, unlike the hydroxyzine and other first generation antihistamines, it penetrates brain poorly and produces negligible/mild sedative effect. Cetirizine also blocks release of histamine from mast cells and may inhibit migration of eosinophils. It has also been shown to inhibit eosinophil chemotaxis and leukotriene B_4 release. Thus it benefits allergic disorders by antihistaminic as well as other actions. It is well absorbed after oral administration and has relatively longer duration of action.

Cetirizine is widely used in human medicine and has been considered in dogs and cats for treatment of pruritus and allergic conditions. However, its use in veterinary medicine is mostly empirical as there are no clinical studies to document its efficacy in animals. Sedation and anticholinergic side effects are not likely as with conventional antihistamines. However, with higher doses sedation is still possible.

Dose

Dogs and cats : 1 mg/kg, PO, once daily.

Levocetirizine

Levocetirizine is the active levo-enantiomer of cetirizine racemate. Levocetirizine acts by blocking histamine H_1 receptors and appears to have other actions of cetirizine as well. It does not enter the brain in significant amounts, and is therefore unlikely to cause drowsiness. It is used for same allergic conditions as mentioned for cetirizine and is claimed to be more effective with fewer side effects. It is particularly very useful in the treatment of allergic airway inflammations.

2. Piperidines

Terfenadine

Terfenadine is a piperidine non-sedating antihistamine. Terfenadine is basically a prodrug, generally completely metabolised to the active form fexofenadine by the intestinal cytochrome P450. Due to the pre-systemic gut wall metabolism, terfenadine normally is not measurable in the plasma. It has rapid onset (1-2 hours) and moderate duration (12-24 hours) of action. Terfenadine possesses considerable cardiotoxic effect (ventricular arrhythmia), particularly in liver disease or when drug is combined with macrolide antibiotics (e.g. erythromycin) or antifungal agents (e.g. ketoconazole) which inhibit drug-metabolising enzymes. This cardiotoxic effect is not observed with its active metabolite fexofenadine that has been marketed as a separate drug. Because of this risk, popularity of terfenadine has declined and it has been withdrawn from market in several countries.

Dose

Dogs : 0.25 – 2.5 mg/kg, PO, once daily.

Astemizole

Astemizole is another piperidine non-sedating antihistamine, which is having actions similar to those of terfenadine. Astemizole does not cross the blood-brain barrier, and H_1 receptor binding is mostly in the peripheral rather than central nervous system. Astemizole may also act on histamine H_3 receptors, thereby producing some adverse effects. It has slow onset (2- 4 hours) and long duration (2-5 days) of action in human beings. Its efficacy in animals has not been established. It shares the cardiotoxic (ventricular tachycardia) and drug interaction potential of terfenadine. Accordingly, it has been withdrawn from the market in several countries.

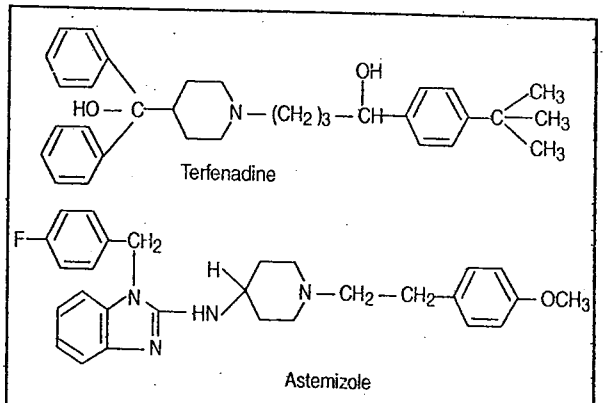


Fig. 27.5. Chemical structures of selected second-generation H_1-receptor antagonists.

)ose
)ogs : 0.2 up to 1 mg/kg, PO, once daily.

exofenadine

Fexofenadine was developed as a successor and alternative) terfenadine. It possesses H_1-receptor blocking activity and is sed in human medicine in the treatment of various allergic onditions including allergic rhinitis and chronic idiopathic urticaria. exofenadine, like other second antihistamines, does not readily oss the blood-brain barrier, and hence causes minimal drowsiness id other side effects. It is preferred over terfenadine and temizole in liver diseases and with drugs like macrolide antibiotics id antifungals.

oratadine

Loratadine is a long acting second-generation piperidine H_1-ceptor antagonist. In addition to H_1 receptor blocking action, ratadine also decreases histamine release from basophiles. It cks CNS depressant effect and is faster acting than astemizole. Like terfenadine, loratadine produces an active metabolite desloratadine (descarboethoxyloratadine) with a longer half-life, but it does not produce cardiac arrhythmia in overdose or show significant interaction with macrolide or antifungal drugs. Although well tolerated, efficacy or safety of loratadine has not been studied in detail in animals.

Desloratadine

Desloratadine is an active metabolite of loratadine, which is also available in market. It has a selective and peripheral H_1-antagonist action with a long duration of action. It does not cause sedation and drowsiness because it does not readily enter the central nervous system. It is mainly indicated in human medicine for the treatment of allergies.

Mizolastine

Mizolastine is a second generation non-sedating antihistamine. It blocks H_1 receptors and is commonly fast-acting drug. It is effective in allergic rhinitis and urticaria. Unlike azelastine, it has no

Table 27.2: Pharmacological profile of selected H_1-receptor antagonists.

Drug	Potency	Sedative effect	Anti-muscarinic effect	Anti-emetic effect	Other effect(s)
First-Generation H_1-Antagonists					
1. Ethanolamines					
Diphenhydramine	++	+++	+++	++ to +++	Local anaesthetic & antitussive.
Clemastine	+ to ++	++	+++	++	
Dimenhydrinate	++	++	++	–	Antitussive.
2. Ethylenediamines					
Mepyramine	+	++	++	–	
Tripelennamine	++	++	+	–	
3. Alkylamines					
Chlorphenamine	+++	+	++	–	
Pheniramine	++	+	+	–	
4. Piperazines					
Hydroxyzine	++	+++	+	+ to ++	Local anaesthetic and tranquillising.
Meclozine	++	+	+	+++	
Cyclizine	++	+	+	+++	
Chlorcyclizine	++	+	+	+++	
5. Phenothiazines					
Promethazine	+++	+++	+++	+++	
Alimemazine	++ to +++	++	+++	+++	Antitussive.
6. Miscellaneous					
Cyproheptadine	++	+	++	–	Anti-serotonin and C^{++} channel channel blocking.
Phenindamine	++	±	++	–	
econd-Generation H_1-Antagonists					
1. Piperazines					
Cetirizine	++ to +++	±	±	–	Inhibition of histamine release and eosinophil migration.
2. Piperidines					
Terfenadine	++ to +++	±	–	–	Inhibition of histamine release.
Astemizole	++ to +++	±	±	–	
Loratadine	++ to +++	±	±	–	
3. Miscellaneous drugs					
Azelastine	+++	–	–	–	Inhibition of histamine release and down regulation of intra-cellular adhesion molecule-1.

mbol: +++ = high; ++ = moderate; + = low; ± = low to none; – = none.

active metabolite, but still it is a long acting drug requiring single daily dosing. Side effects can include dry mouth and throat.

Ebastine

Ebastine is a new second-generation H_1 receptor antagonist that is indicated mainly for allergic rhinitis and chronic idiopathic urticaria. It is non-sedating because it does not penetrate the blood-brain barrier and hence blocks only peripheral H_1-receptors. After oral administration, ebastine undergoes extensive first-pass metabolism by hepatic cytochrome P450 isozymes into its active carboxylic acid metabolite, carebastine. Ebastine is often available in micronized form, due to poor water solubility.

3. Miscellaneous Drugs

Azelastine

Azelastine is a newer potent second generation H_1-receptor antagonist with good topical activity. In addition to H_1-blocking action, azelastine inhibits histamine release from mast cells and inflammatory reaction triggered by leukotrienes and platelet activating factor. After intranasal administration, it has been shown to down regulate intracellular adhesion molecule-1 (ICAM-1) expression on nasal mucosa. The systemic bioavailability of azelastine is approximately 40% when administered intranasally. Azelastine is metabolized by the cytochrome P-450 family into an active metabolite, desmethylazelastine, and two inactive carboxylic acid metabolites. Azelastine has a rapid onset of action; 15 minutes with the nasal spray and 3 minutes with the eye drops. The elimination half-life of azelastine is about 24 hours in humans, but effects last longer to active metabolite.

Azelastine is used topically either as nasal spray (0.1% and 0.15% solutions) or eye drops (0.05% solution). Azelastine nasal spray is indicated for the local treatment of the symptoms of allergic rhinitis such as rhinorrhoea, sneezing and nasal pruritus. Azelastine eyes drops are indicated for the local treatment of seasonal and perennial allergic conjunctivitis. Stinging in the nasal mucosa and altered taste perception have been noted as side effects in humans. Azelastine nasal spray has a more rapid onset of action and is more effective than oral antihistamines such as cetirizine or desloratadine in the treatment of allergic rhinitis.

Other drugs

Mequitazine, acrivastine, rupatadine and olopatadine are some other H_1-receptor blockers available for clinical use in human medicine. Mequitazine is a phenothiazine derivative while acrivastine is an alkylamine antihistamine; both are used to treat allergies and rhinitis. Rupatadine is a recently introduced second generation antihistamine with additional platelet activating factor (PAF) blocking action (see also Chapter 29), used to treat allergies. Olopatadine is an antihistamine, anticholinergic and mast cell stabilizer, used mainly as an eye drop (0.2% solution) to treat itching associated with allergic conjunctivitis.

B. H_2-RECEPTOR ANTAGONISTS

The H_2-Receptor antagonists selectively block H_2-receptors and antagonise the histamine effect on gastric secretion. These drugs are used primarily in the gastric hypersecretory states and peptic ulcers and are discussed in Chapter 42.

C. H_3 AND H_4 RECEPTORS ANTAGONISTS

Although specific H_3 and H_4 receptor antagonists have been developed, no drugs have been approved for clinical use.

SEROTONIN

Serotonin (5-Hydroxytryptamine, 5-HT) is a biogenic amine, which is widely distributed in animal and plant kingdoms. In the animal body, serotonin is primarily found in the gastrointestinal tract, platelets and central nervous system. Serotonin acts as a neurotransmitter in the central nervous system and plays important roles in the regulation of smooth muscle function in gastrointestinal and cardiovascular systems, and in platelet formation. Accordingly, it is also called enteramine or vasotonin. Serotonin itself has no therapeutic value, but is important with respect of action of other drugs and diseases and some of its antagonists.

Chemistry and Distribution

Serotonin is 3-(β-aminoethyl)-5-hydroxyindole. Like histamine, serotonin is widely distributed in animals and plants. In vertebrates, about 80% of the total body serotonin is present in the enterochromaffin cells of GI tract with highest concentration in the duodenum. Most of the remainder serotonin is found in platelets and in specific region of the CNS. As a component of penial gland, it functions as a precursor of melatonin. In addition to animals, serotonin is found in several insects, fungi, plants and pathogenic amoebas. Its presence in insect venoms and plant spines is responsible for pain and toxic effects.

Synthesis, Storage, Release and Metabolism

Synthesis, storage and release: Serotonin is synthesised mainly in the enterochromaffin cells of GI tract and in the serotonergic neurons of CNS from dietary amino acid l-tryptophan in a two-step chemical reaction. In the first step, l-tryptophan is hydroxylated by the enzyme tryptophan-5-hydroxylase (TPH) to give 5-hydroxytryptophan (5-HTP), which is then decarboxylated by l-aromatic acid decarboxylase to form 5-hydroxytryptamine or serotonin (Figure 27.6). The TPH-mediated reaction is the rate-limiting step in the pathway. The l-amino acid decarboxylase (also called 5-HTP decarboxylase or DOPA decarboxylase) enzyme is widely distributed in body and is a non-specific enzyme because it is involved also in the synthesis of norepinephrine.

After synthesis, serotonin is either stored in the storage sites or rapidly inactivated by metabolism. The serotonin is often stored in neurons and chromaffin cells together with various peptide hormones such as somatostatin, substance P and vasoactive intestinal polypeptide (VIP). Most of the storage of serotonin in body occurs in the enterochromaffin cells, which is also the source of circulating serotonin. Serotonin released from enterochromaffin cells enters the portal vein and is subsequently metabolised mostly in the liver. Platelets do not synthesise serotonin due to lack of decarboxylase enzyme, but collect the autacoid from blood by an active transport mechanism and store it in high concentrations in secretory granules. Mast cells of some species (e.g. rats, mice and bovines) also contain serotonin. It appears that serotonin is also continually produced and destroyed in the pool of intestine and brain. In platelets, it appears to be released only upon their destruction.

Metabolism: Serotonin is mainly degraded through oxidative deamination by monoamine oxidase (MAO) to form 5-hydroxyindoleacetaldehyde, which is then oxidised to the 5-hydroxyindoleacetic acid (5-HIAA). Alternately, reduction of the acetaldehyde to an alcohol 5-hydroxytryptophol can occur, but

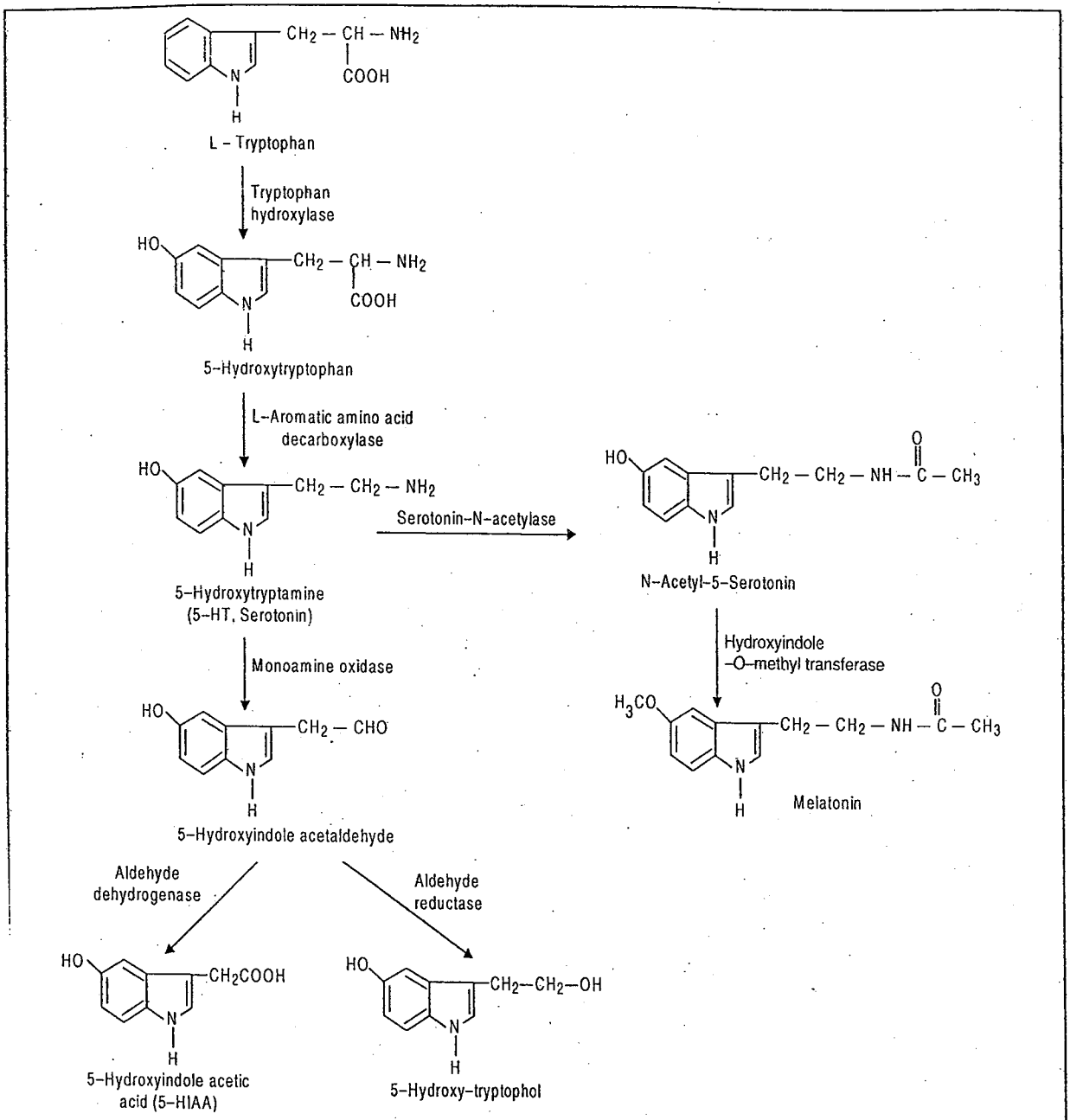


Fig. 27.6. Biosynthesis and metabolism of Serotonin.

his pathway is not important. It can also follow acetylation to N-acetyl-serotonin or methylation to 5-methoxytryptamine. Acetylation followed by methylation converts serotonin to melatonin in the pineal gland. 5-Hydroxyindoleacetic acid, the major metabolite of serotonin, is excreted in urine and serves as an indicator of serotonin production in the body. Serotonin and 5 HIAA are sometimes produced in excess amounts by certain tumours or cancers, and levels of these substances may be measured in the urine to test for these tumours.

In addition to metabolism by MAO, a Na^+-dependent carrier mediated uptake process exists in the CNS, which causes reuptake of serotonin released by nerve impulse and helps in terminating the action of autacoid. The mechanism of synthesis, storage, release and re-uptake of serotonin in CNS are in general similar to those of norepinephrine and hence many drugs affect both processes indiscriminately.

Mechanism of Action

Serotonin has multiple actions in the body, which are mediated through a variety of serotonin receptors, abbreviated as 5-HT receptors. These receptors are located on the cell membrane of nerve cells and other cell types in animals, and mediate the effects of serotonin as the endogenous ligand and of a broad range of pharmaceutical and hallucinogenic drugs.

The serotonin 5-HT receptors have been categorised on the basis of pharmacological characterisation and cloning of receptors into seven types (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7) comprising of total 14 receptor subtypes (see Table 27.3). The multiple serotonin receptor subtypes cloned today are the largest of all known autacoids or neurotransmitter receptor family. These receptors have distinct but often overlapping characteristics and are coupled to different transmembrane-signalling mechanisms. With the exception of the 5-HT_3 receptor, all other serotonin receptors

Table 27.3 Serotonin receptor types and subtypes.

Sr. No.	Receptor Type	Receptor subtypes
1.	5-HT_1	A, B, D, E, F
2.	5-HT_2	A, B, C
3.	5-HT_3	–
4.	5-HT_4	–
5.	5-HT_5	A, B
6.	5-HT_6	–
7.	5-HT_7	–

are G protein-coupled receptors which activate an intracellular second messenger cascade to produce an excitatory or inhibitory response. The 5-HT_3 is an ionotropic receptor (ligand gated ion channel) that directly regulates Na^+ and K^+ channels. Of various receptor types, only 5-HT_1 to 5-HT_4 have distinct functions.

5-HT_1 receptors: Five subtypes of 5-HT_1 receptor viz. 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} have been identified. The 5-HT_{1C} designation is vacant, as the receptor was renamed 5-HT_2C, due to structural, operational, and transductional similarities with the 5-HT_2 receptor family. All the five receptor subtypes are coupled to G_i/G_o-protein and are present mainly in the CNS (all subtypes) and cranial blood vessels (5-HT_{1D}). All subtypes of 5-HT_1 receptor inhibit adenylate cyclase enzyme; 5-HT_{1A} also activate a receptor-operated K^+ channel and inhibits a voltage gated Ca^{++} channel. These receptors are present mainly pre-synaptically and function primarily as autoreceptors in brain inhibiting firing of serotonergic neurons or release of serotonin from nerve endings. The 5-HT_{1A} receptor is found in the raphe nuclei of the brainstem, where it functions as an inhibitory autoreceptor on cell bodies of serotonergic neurons and the 5-HT_{1D} receptor, functions as an autoreceptor on axon terminals inhibiting 5-HT release. The 5-HT_{1D} receptors, present mainly in substantia nigra and basal ganglia, also regulate the firing of dopamine-containing cells and release of dopamine at axonal terminals, and cause constriction of cranial blood vessels.

The anti-anxiety drug buspirone functions as a serotonin 5-HT_{1A} receptor partial agonist and antimigraine drug sumatriptan is a selective 5-HT_{1D} agonist.

5-HT_2 receptors: Three subtypes of 5-HT_2 receptor viz. 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} have been identified, which are linked through G_q/G_{11}-protein to phospholipase C with the generation of second messengers diacylglycerol and inositol triphosphate. The 5-HT_{2A} receptor subtype also inhibits K^+ channels resulting in slow depolarisation of neurons. 5-HT_2 receptors are located in CNS and also in many peripheral sites like vascular and visceral smooth muscles, platelets and autonomic sites. The 5-HT_{2A} is the most widely expressed post-synaptic serotonin receptor and it mediates actions like vasoconstriction, intestinal and uterine contraction, platelet aggregation and activation of cerebral neurons. 5-HT_{2C} receptor is located on vascular endothelium and elicits vasodilatation through endothelium dependent relaxing factor (EDRF). The role of 5-HT_2 receptors in physiological process is probably a minor one, but it becomes prominent in pathological conditions like asthma and vascular thrombosis.

5-HT_3 receptors: The 5-HT_3 is the only monoamine neurotransmitter receptor that is known to function as a ligand-gated ion channel. It occurs mainly in the peripheral nervous system, especially in nociceptive afferent nerves and on autonomic and enteric neurons, on which serotonin exerts a strong excitatory effect (pain, itch and other visceral reflexes). The 5-HT_3 receptors also occur in brain, especially in nucleus tractus solitari and in area postrema. 5-HT_3 receptors in both the gastrointestinal tract and the CNS participate in the emetic responses.

2-Methyl-serotonin is a selective 5-HT_3 agonist and ondansetron is a selective 5-HT_3 antagonist.

5-HT_4 receptors: The 5-HT_4 receptors are widely distributed throughout the body. These are coupled to G_s-protein and activate adenylate cyclase enzyme leading to a rise in intracellular cAMP. In the GI tract, 5-HT_4 receptors located on the neurons (e.g. myentric plexus), smooth muscles and secretory cells are thought to be involved in augmenting intestinal secretion and peristalsis (see also Chapter 43). In the CNS, the receptors are found mainly on neurons of the hippocampus and the colliculi, where they cause hyperpolarisation by decreasing K^+ conductance.

Cisapride is a selective 5-HT_4 agonist and piboserod is a selective antagonist.

Other serotonin receptors: Three other serotonin receptor types include 5-HT_5, 5-HT_6 and 5-HT_7. Two subtypes of the 5-HT_5 receptor namely 5-HT_{5A} and 5-HT_{5B} have been cloned and found to exist in different species. Although the 5-HT_{5A} receptor is coupled to G_i/G_o-protein and acts by inhibition of adenylate cyclase enzyme, the functional coupling of the cloned 5-HT_{5B} receptor has not yet been described. The 5-HT_6 and 5-HT_7 subtypes are coupled to G_s-protein and function through activation of adenylate cyclase enzyme. The physiological functions of these receptor types have not been recognised.

Clozapine, an atypical neuroleptic, has high affinity for 5-HT_6 and 5-HT_7 receptors and act as their antagonist.

Physiological and Pharmacological effects

The actions of serotonin are numerous and complex, and show considerable species variations.

Cardiovascular system:

Blood vessels: Effect of serotonin on blood vessels varies according to the size of vessel and the prevailing sympathetic activity. Large vessels, both arteries and veins, are usually constricted by direct action (mediated via 5-HT_{2A}) of 5-HT, though sensitivity varies (pulmonary, renal and cerebral vessels are most sensitive). In the microcirculation, serotonin dilates arterioles and constricts venules resulting in increased capillary pressure and escaping of fluid from the capillaries. Arterioles are dilated by several mechanisms, all mediated through 5-HT_1 receptors. These include release of nitric oxide and prostaglandins which relax vascular smooth muscle, blockade of norepinephrine release from sympathetic nerves that decreases sympathetic tone, and possibly a direct relaxant effect of serotonin on vascular smooth muscle.

Blood pressure: Serotonin is not involved in the physiological regulation of blood pressure. However, IV injections of serotonin in animals produces a triphasic response on blood pressure i.e. an early fall, then brief rise, followed by a prolonged fall. The early depressor phase is due to stimulation of coronary chemoreceptors (Bezold-Jarisch effect). The middle pressor phase is due to vasoconstriction and increased cardiac output. The late and final depressor phase is due to arteriolar dilation and extravasation of fluids.

Table 27.4: Main serotonin (5-hydroxytryptamine) receptor subtypes with important features.

Feature	5-HT_1		5-HT_2			5-HT_3	5-HT_4
	5-HT_{1A}	5-HT_{1D}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}		
Distribution	CNS	CNS, cranial blood vessels.	CNS, PNS, smooth muscle, platelets.	Gastric fundus.	CNS, choroid plexus.	PNS, CNS (Area postrema).	PNS (GI tract), CNS.
Main effects and function	*Autoreceptor:* Neuronal inhibition, behavioural effects.	Cerebral vasoconstriction, behavioural effects.	Neuronal excitation, platelet aggregation, smooth muscle contraction, vasoconstriction.	Contraction.	CSF secretion.	Neuronal excitation; emesis.	GI motility, neuronal excitation.
Receptor type	G-Protein coupled (G_i/G_o-protein).	G-protein coupled (G_i/G_o-protein).	G-protein coupled (G_q/G_{11}-protein).	G-Protein coupled (G_q/G_{11}-protein).	G-Protein coupled (G_q/G_{11}-protein).	Ionotropic receptor.	G-Protein coupled (G_s-protein).
Signal transduction	Inhibition of AC.	Inhibition of AC.	Activation of PLC.	Activation of PLC.	Activation of PLC.	Ligand-operated ion channel ($\uparrow Na^+$ and K^+ channel).	Activation of AC.
Second messenger	$\downarrow$ cAMP	$\downarrow$ cAMP	$\uparrow$ IP_3/DAG	$\uparrow$ IP_3/DAG	$\uparrow$ IP_3/DAG	–	$\uparrow$ cAMP
Selective agonist	8-OH-DPAT	Almotriptan, sumatriptan.	–	Norfenfluramine	Lorcaserin	2-Me-5-HT	Cisapride, mosapride.
Selective antagonist	Lecozotan	–	Ritanserin	–	Agomelatine	Ondansetron, tropisetron.	Piboserod

Abbreviations: CNS = central nervous system; PNS = peripheral nervous system; CSF = cerebrospinal fluid; AC = adenylate cyclase; cAMP = cyclic 3',5'-adenosine monophosphate; IP_3 = inositol 1,4,5-triphosphate; DAG = diacyl glycerol; GI gastrointestinal; 2 Me-5 HT = 2 methyl 5-hydroxytryptamine; 8-OH-DPAT = 8-hydroxy-2-(dipropylamino)-tertraline.
$\uparrow$ = Increase; $\downarrow$ = decrease.

Heart: Serotonin has diverse actions on the heart. It produces weak positive inotropic and chronotropic effects on heart (often seen in an isolated heart) both directly (5-HT_1) and through release of norepinephrine from nerve endings. In intact animal, the cardiac stimulant effect is usually masked by reflex bradycardia caused by activation of coronary chemoreflex through action on vagal afferent nerve endings in coronary bed.

Smooth muscles:

Gastrointestinal tract: Serotonin causes increase in gastrointestinal motility partly by a direct action on GI smooth muscles (5-HT_{2A} in intestine and 5-HT_{2B} in gastric fundus) and partly due to an indirect excitatory effect at nerve endings on enteric muscle (5-HT_4) and at post-synaptic cells of enteric ganglia (5-HT_3). Release of enteric serotonin is augmented by mechanical stretching (as caused by food), vagal stimulation (response to ACh), lowered pH, etc. Presence of 5-HT_3 receptors on vagal and other afferent neurons and on enterochromaffin cells play important role in emesis.

Other smooth muscles: Serotonin contracts various smooth muscles like uterus and bronchi. However, these effects are species dependent and are less significant than those of histamine.

Platelets: Serotonin causes change in the shape of platelets and is a weak aggregator of platelets through 5-HT_{2A} receptors. The aggregated platelets which collect in the vessel in turn release more 5-HT. If the endothelium is intact, serotonin released from adherent platelets causes vasodilatation and helps to sustain blood flow. On the other hand, if the vascular smooth muscle is exposed due to injury, serotonin directly constricts the vessel and promotes haemostasis.

Central nervous system: Serotonin is an important neurotransmitter and also a neuromodulator in the CNS. Its actions in the CNS are either excitatory or inhibitory depending on the receptor subtypes. In general, serotonin influences a multitude of brain functions including sleep, cognition, sensory perception, motor activity, thermoregulation, appetite, nociception, vomiting, sexual behaviour, mood and emotions, and hormone secretions. It is likely that multiple serotonin receptor subtypes with similar or opposing actions are expressed in individual neurons leading to tremendous diversity of actions. Serotonin found in the pineal gland is believed to serve essentially as a precursor for the synthesis of melatonin. Exogenously administered serotonin does not produce central effects as it poorly crosses the blood brain barrier.

Other effects: Serotonin inhibits secretion of pepsin and acid, but increases mucus production. Effect on other glandular secretion is not significant. Serotonin stimulates adrenal medulla and releases catecholamines, but its effect is less potent than that of histamine. The serotonin is a potent stimulant of nociceptive (pain-mediating) sensory nerve endings. If injected into the skin, it causes pain and itching.

Patho-Physiological Roles

Serotonin is involved in a number of physiological and pathological functions.

1. ***Neurotransmitter:*** Serotonin acts as a neurotransmitter in the CNS and is believed to be involved in a number of brain functions (see for detail Central Neurotransmitters, Chapter 17). Mood disorders and anxiety are related to disturbed serotonin functions. Many important antidepressant and antianxiety drugs mediate their action through serotonin mechanisms.
2. ***Inflammation and allergy:*** Serotonin is involved in inflammatory reactions, allergy and anaphylactic reactions. However, unlike histamine, it is not a strong mediator.
3. ***Gastrointestinal function:*** Serotonin is involved in the regulation of peristalsis and local reflexes in gut through activation of local receptors. Some prokinetic agents such as cisapride mediate GI their action through serotonin receptors.
4. ***Nausea and vomiting:*** Serotonin is involved in the nausea and vomiting, particularly that evoked by cytotoxic drugs or radiotherapy. These effects are mediated through 5-HT_3 receptors in gut, area postrema and nucleus solitaries. Some 5-HT_3 antagonists, such as ondansetron and granisetron are important antiemetic agents (see for detail Chapter 44).
5. ***Haemostasis:*** Serotonin is involved in the haemostasis through platelet aggregation and vasoconstriction (see for detail Chapter 35).
6. ***Migraine:*** Serotonin is said to initiate the vasoconstrictor phase of migraine in human beings. It also participates in neurogenic inflammation of the affected blood vessels. Consistent with the hypothesis that serotonin is a key mediator in the pathogenesis of migraine, 5-HT receptor have become the main target of several drugs such as sumatriptan for the acute treatment of migraine headaches.
7. ***Carcinoid syndrome:*** Carcinoid syndrome (tumour of enterochromaffin cells) secretes large amount of serotonin. This causes bowel hypermotility, colic, episodes of bronchospasm and hyperventilation. The condition is often diagnosed by estimating 5-HIAA level in the urine and serotonin level in the blood.

PHARMACOLOGY OF SEROTONIN SYSTEM

Serotonin itself has no therapeutic or diagnostic uses, but several agents have been found that affect serotonin system in the body. Many of these compounds have been useful as experimental tools for manipulating amount of serotonin in tissues and some of the agents are now available for clinical use. These drugs affect synthesis, release, storage, uptake, metabolism and/or action of serotonin in body.

A. Drugs Affecting Synthesis of Serotonin

Tryptophan

Tryptophan is an essential amino acid and a precursor of serotonin. Serotonin taken orally does not pass into the serotonergic pathways of the central nervous system, because it does not cross the blood-brain barrier. However, tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), from which serotonin is synthesized, can cross the blood-brain barrier. These agents are available as dietary supplements and may be effective serotonergic agents. A diet low or rich in tryptophan concentration will also decrease or increase concentration of serotonin in the brain, respectively.

Para-chlorophenylalanine

Para-chlorophenylalanine (PCPA, fenclonine) is a synthetic amino acid which acts as a selective and irreversible inhibitor of tryptophan hydroxylase, which is a rate-limiting enzyme in the biosynthesis of serotonin. The administration of PCPA thus decreases synthesis of serotonin and reduces its actions, acting as an indirect serotonin antagonist. It is not used clinically because it produces profound and long-lasting depletion of serotonin levels in the body. The drug has no effect on the levels of catecholamines. PCPA is used in scientific research to investigate the effects of serotonin depletion on behaviour.

B. Drugs Affecting Release of Serotonin

Halogenated amfetamines

Para-chloroamfetamine (PCA), also known as 4-chloroamfetamine (4-CA), is an amfetamine derivative and a potent monoamine releaser. The PCA and some other halogenated amfetamines (e.g. fenfluramine, dexfenfluramine) promote release of serotonin from platelets and neurons. The profound and unrestrained release of serotonin (also dopamine) by these halogenated amfetamines causes prolonged decline in serotonin concentrations in the brain with subsequent neurotoxicity. PCA has no therapeutic use but is employed as a neurotoxin by neurobiologists to selectively kill serotonergic neurons for research purposes. Fenfluramine and dexfenfluramine are occasionally used in human medicine for its anorectic properties (to reduce appetite).

C. Drugs Affecting Storage/Uptake of Serotonin

Selective Serotonin Re-Uptake Inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are a class of compounds which increase the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cell. This increases level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor. They have also varying degrees of selectivity for the other monoamine transporters, with pure SSRIs having only weak affinity for the norepinephrine and dopamine transporter. The SSRIs such as fluoxetine and trazodone are widely used as antidepressants in the treatment of depression, anxiety disorders and some behaviour abnormalities. The pharmacology of SSRIs has been given in Chapter 23.

Reserpine

Reserpine is an indole alkaloid, obtained from *Rauwolfia serpentina* or Indian snakeroot plant. Reserpine has multiple actions in the body including inhibition of serotonin storage into the synaptic vesicles by causing blockade of vesicular monoamine transporter (VMAT). Reserpine also causes depletion of serotonin from the serotonergic neurons and enhances its metabolism. The effect of reserpine, however, is non-selective as it affects the storage and release of other biogenic amines including norepinephrine and dopamine. The mechanism of action and pharmacology of reserpine have been discussed in Chapter 12.

D. Drugs Affecting Metabolism of Serotonin

Monoamine oxidase inhibitors

Monoamine oxidase (MAO) inhibitors are a class of drugs which selectively block the activity of monoamine oxidase, thus preventing the metabolism of serotonin and other monoamine neurotransmitters, thereby increasing their concentrations in the nervous system. The MAO-inhibitors are primarily used as antidepressant drugs prescribed for the treatment of depression (see for detail Chapter 23). As these drugs also alter concentrations of various catecholamines, so they are less specific for serotonin.

E. Drugs Affecting Action of Serotonin

Several agents affect action of serotonin on its receptor sites either by producing action similar to that of serotonin (serotonin receptor agonists) or blocking action of serotonin on its receptor sites (serotonin receptor antagonists). Many of these agents are non-selective in their action, but some act selectively on various serotonin receptor subtypes. Many of these serotonin receptor acting drugs are now available for therapeutic use in human and veterinary medicines.

I. SEROTONIN RECEPTOR AGONISTS

1. Non-Selective Serotonin Receptors Agonists

Metoclopramide

Metoclopramide is a non-selective drug that acts on dopamine as well as serotonin receptors. On serotonin receptors it acts as a mixed 5-HT_3 receptor antagonist/5-HT_4 receptor agonist. Metoclopramide is commonly used to treat nausea and vomiting (emesis) associated with conditions including emetogenic drugs, uremia, radiation sickness, malignancy and infection It is also used as a prokinetic drug and is considered useful in the treatment of gastric stasis (see for detail Chapter 44).

D-Lysergic Acid Diethylamide (LSD)

D-Lysergic acid diethylamide (LSD), a semi-synthetic ergot derivative and a potent hallucinogen, is a non-selective serotonin receptors agonist or partial agonist. It activates many subtypes of serotonin receptors including 5-HT_{1A}, 5-$HT_{2A/2C}$ and 5-HT_{5-7} in specific brain areas. However, it antagonises 5-HT_{2A} receptors in the ileum. LSD also affects a large number of other receptors, including all dopamine receptor subtypes and all adrenoreceptor subtypes. A number of other hallucinogen drugs act as partial or full agonist at brain serotonin receptors. LSD has no clinical application in human or veterinary species.

Psilocin

Psilocin (4-Hydroxy-dimethyltryptamine, 4-HO-DMT), is a psychedelic (hallucinogenic) mushroom alkaloid. Psilocin is structurally similar to serotonin, differing only by the hydroxyl group being on the 4-position rather than the 5 and the dimethyl groups on the nitrogen. It acts as a $5HT_{2A}$, $5HT_{2C}$ and $5HT_{1A}$ agonist. Psilocin has no significant effect on dopamine receptors, but may affect the noradrenergic system at very high dosages. Psilocin is a Schedule I drug under the Convention on Psychotropic Substances and has no therapeutic uses.

2. Selective Serotonin Receptor Agonists

Azapirones

Azapirones like buspirone, gepirone, isapirone and tandospirone are a new class of antianxiety drugs, which act as partial agonist of 5-HT_{1A} receptors in the brain. These drugs are useful for the treatment of anxiety related behaviour problems in animals. These drugs are also commonly employed as augmentations to antidepressants like the selective serotonin reuptake inhibitors (SSRIs). Pharmacology of azapirones is given in detail in Chapter 23.

कृपया रेषा मारुन
पुस्तक खराब करु नये.

Triptans

Triptans are a family of tryptamine-based drugs used in the treatment of migraines and cluster headaches in human beings. Triptans include sumatriptan, almotriptan, avitriptan, eletriptan, frovatriptan, naratriptan, rizatriptan and zolmitriptan. Sumatriptan was the first triptan available and is the prototype drug. The anti-migraine action of triptans is attributed primarily to their binding to serotonin 5-HT_{1B} and 5-HT_{1D} receptors in cranial blood vessels causing their constriction and subsequent inhibition of pro-inflammatory neuropeptide release. These drugs have no/negligible use in veterinary practices.

Benzamides

A number of substituted benzamides are clinically used mainly as anti-emetic and/or prokinetic agents. Some benzamides such as cisapride, mozapride and prucalopride mediate their action through selective 5-HT_4 receptor stimulation and are used primarily as prokinetics. Some other agents such as dazopride and renzapride act as a 5-HT_4 receptor agonist and a 5-HT_3 receptor antagonist and possess both anti-emetic and prokinetic effects. These drugs are used in both human and veterinary medicines for the treatment of various GI tract related conditions including constipation, irritable bowel syndrome, feline megacolon and emesis. The pharmacology of some of these drugs is given in Chapters 43 and 44.

8-OH-DPAT

8-OH-DPAT (8-Hydroxy-(2-N,N-dipropylamino)-tertraline) is a research chemical of the aminotetralin chemical class which was developed in the 1980s and has been widely used to study the function of the 5-HT_{1A} receptor. It was one of the first major 5-HT_{1A} receptor agonists to be discovered. Originally believed to be selective for the 5-HT_{1A} receptor, 8-OH-DPAT was later found to act as a 5-HT_7 receptor agonist and serotonin reuptake inhibitor/releasing agent as well. 8-OH-DPAT reduces the firing rate of raphe cells by activating 5-HT_{1A} autoreceptors and inhibits neuronal firing in terminal fields (e.g. hippocampus) by direct interaction with postsynaptic 5-HT_{1A} receptors.

Norfenfluramine

Norfenfluramine is a psychoactive drug which functions as a $5HT_{2B}$ receptor agonist and a potent serotonin releasing agent. Similar to other halogenated amfetamines (e.g. fenfluramine and dexfenfluramine), it was initially used as an anorectic drug, but its action on $5HT_{2B}$ receptors on heart valves leading to a characteristic pattern of heart failure following proliferation of cardiac fibroblasts on the tricuspid valve, known as cardiac fibrosis, led to its withdrawal from market.

Lorcaserin

Lorcaserin is a newly developed anorectic and weight-loss drug. It is structurally similar to dexfenfluramine, the halogenated amfetamine, but acts as a selective 5-HT_{2C} receptor agonist. The activation of 5-HT_{2C} receptors in the hypothalamus is supposed to activate proopiomelanocortin (POMC) production and consequently promote weight loss through satiety. Lorcaserin is presently undergoing last phases of clinical trial.

II. SEROTONIN RECEPTORS ANTAGONISTS

1. Non-Selective Serotonin Receptor Antagonists

Ergot Alkaloids

Ergot alkaloids (e.g. ergotamine) and related compounds (e.g. methysergide) act as partial agonists and antagonists at serotonin receptors. Their actions are complex and diverse (see also Chapter 12). Ergotamine, in addition to serotonin receptors, acts as a partial agonist and antagonist at α-adrenergic receptors and is used mainly for the treatment of migraine in human beings. Methysergide is used in the symptomatic treatment of carcinoid syndrome and in the prophylaxis of migraine. The therapeutic effect of methysergide in migraine is believed due to its antagonistic action at the 5-HT_{2B} receptor. Furthermore, it is also an antagonist at the 5-HT_{2C} receptor and at the 5-HT_{1A} receptor. It is known to have partial agonist effects on some of the other 5-HT receptors as well.

Atypical antipsychotics

Several atypical antipsychotics such as clozapine, olanzapine, quetiapine, risperidone and ziprasidone used in humans for treatment of schizophrenia and bipolar disorder possess significant serotonin receptor blockade activity. Clozapine has non-selective action at dopamine and serotonin receptors with an antagonist/inverse agonist at 5-HT_{2A} and 5-HT_{2C} receptors, and some partial agonist at the 5-HT_{1A} receptor. Olanzapine has a higher affinity for 5-HT_2 serotonin receptors than D_2 dopamine receptors; it also exhibits low affinity for histamine, cholinergic muscarinic and alpha-adrenergic receptors. Quetiapine is a serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7), dopamine, adrenergic and histamine receptors antagonist. Risperidone has high affinity for D_2 dopaminergic receptors with significant blocking action at some serotonin receptor subtypes (5-HT_{2C} and 5-HT_{2A}). Ziprasidone has a high to moderate affinity for dopamine, serotonin (5-HT_{1A} agonist, 5-HT_{1D} antagonist and 5-HT_{2A} antagonist/inverse agonist), alpha-adrenergic and histamine receptors with additional action as inhibitor of serotonin and norepinephrine re-uptake transporter (SERT and NET).

Phenothiazines

Phenothiazines (e.g. chlorpromazine) are an important group of neuroleptic and behaviour modifying drugs which non-selectively block dopamine, α-adrenergic, serotonin and histamine receptors. On serotonin system, their antagonistic actions on 5-HT_1 and 5-HT_2 are responsible for anxiolytic and antiaggressive effects. The pharmacology of phenothiazines is discussed in Chapter 21.

Beta-adrenoceptor blockers

Some selective and non-selective beta-adrenoceptor blockers, including alprenolol, pindolol, cyanopindolol, iodocyanopindolol and oxprenolol possess significant beta receptor blocking as well as 5-HT_{1A} receptor antagonist activities. The clinical usefulness of anti-serotonin action of these drugs is not clear.

Cyproheptadine

Cyproheptadine is a combined serotonin (mainly 5-HT_{2A}), histamine (H_1) and cholinergic antagonists. Its anti-serotonin (mainly 5-HT_2 receptor antagonist) activity has been utilised in the management of serotonin syndrome and in the symptomatic treatment of carcinoid syndrome. Cyproheptadine has also been used in the treatment of cyclical vomiting syndrome and to stimulate the appetite. The pharmacology of cyproheptadine has been discussed earlier in the present chapter (See antihistamines).

Yohimbine

Yohimbine, the principal alkaloid of the bark of the West African evergreen *Pausinystalia yohimbe* and an alpha$_2$-

adrenoceptor blocker, possesses weak to moderate antagonistic activity at dopamine D_2 and serotonin 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2B} receptors, and partial agonistic action at serotonin 5-HT_{1A} receptors. Its antagonistic actions at some serotonin receptors might be contributing in its application for the treatment of sexual side effects caused by some selective serotonin re-uptake inhibitor (SSRI) antidepressants.

Ketanserin

Ketanserin is a substituted quinazoline having 5-HT_2 receptor blocking property with negligible action on other serotonin receptor types. Among 5-HT_2 receptor subtypes, ketanserin potently blocks 5-HT_{2A} and 5-HT_{2C} receptors. Ketanserin also has high affinity for alpha$_1$ adrenergic receptors, and very high affinity for histamine H_1 receptors. In addition, it has weak affinity for dopamine D_1 and D_2 receptors. Ketanserin lowers blood pressure in hypertensive patients and its effect is likely related to the blockade of alpha-$_1$ adrenergic receptors and not to the blockade of 5-HT_{2A} receptors. Ketanserin also inhibits 5-HT-induced platelet aggregation, but it does not greatly reduce the capacity of other agents to cause aggregation. The drug is presently marketed as antihypertensive drug in human medicine.

2. Selective Serotonin Receptor Antagonists

Ritanserin

Ritanserin is a new drug related to ketanserin. However, it is more selective for 5-HT_{2A} and less for 5-HT_{2C} receptor. Unlike ketanserin, it possesses low affinity for alpha$_1$-adrenergic receptors. It is used together with typical antipsychotics in the treatment of schizophrenia.

Ondansetron and related drugs

Ondansetron, granisetron, tropisetron and dolasetron are selective 5-HT_3 receptor antagonists which are approved for use in the prevention of nausea and vomiting associated with cancer chemotherapy. These drugs do not have any effect on dopamine receptors or muscarinic receptors and have as such not much effect on vomiting due to motion sickness. The pharmacology of these drugs has been discussed in Chapter 44.

Agomelatine

Agomelatine is an antidepressant that acts as a melatonergic MT_1 and MT_2 receptor agonist and serotonin 5-HT_{2C} receptor antagonist. It appears to have no affinity for adrenergic, histaminergic, cholinergic, dopaminergic and benzodiazepine receptors. Agomelatine is indicated for the treatment of major depressive disorder in human medicine.

Lecozotan

Lecozotan is an investigational drug that selectively antagonizes 5-HT_{1A} receptor, which enhances the potassium-stimulated release of acetylcholine and glutamate. Presently, it is undergoing clinical trials for improvement of cognitive functions of Alzheimer's disease in human patients.

Piboserod

Piboserod is a highly and potently selective 5-HT_4 receptor antagonist. It is developed for the management of serotonin 5-HT_4 receptor atrial fibrillation and irritable bowel syndrome. Presently it is undergoing clinical trials.

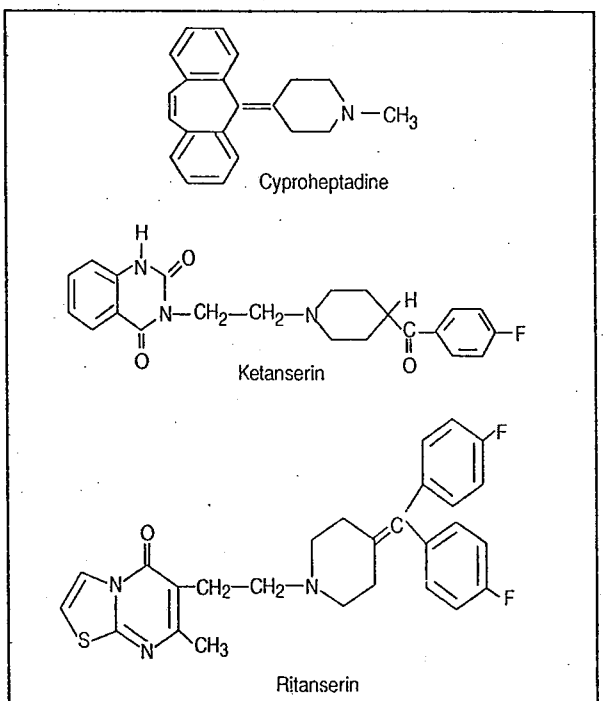


Fig. 27.7. Chemical structures of selected 5-HT-receptor antagonists.

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CHAPTER 28 PEPTIDE AUTACOIDS

Kinins
Bradykinin and kallidin
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- Synthesis and metabolism
- Mechanism of action
- Physiological and pharmacological effects
- Pathophysiological roles
- Pharmacology of kinins
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Tachykinins
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  - NK_1-receptor antagonists
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Cytokines

Various types of peptide/polypeptide autacoids exist in body which exert important effects on blood vessels and smooth muscles, and possibly perform important regulatory functions in the body. Some of these peptide autacoids are involved also in tissue damage, allergic reactions, viral infections and other inflammatory conditions. The important and well established peptide autacoid groups include kinins (bradykinin and kallidin), angiotensins, tachykinins and cytokines. In addition, there are many other peptide substances in the body, whose role in the physiological processes in the body as autacoids is still in the process of being defined.

KININS

The kinins are a group of structurally and physiologically related peptide autacoids which are involved mainly in vasodilatation, increased vascular permeability, nociception and synthesis of prostaglandins. Among them, bradykinin and kallidin are of greatest importance.

BRADYKININ AND KALLIDIN

Bradykinin (Greek words: *bradys* = slow, *kinin* = to move) and kallidin are vasodilator peptide autacoids which are generally referred to as plasma kinins. Both autacoids originate from the same circulating $alpha_2$ globulins (kininogens) and have effects somewhat similar to those of histamine on vascular smooth muscle, capillary permeability, and bronchial and intestinal smooth muscles.

Chemistry and Distribution

Bradykinin is a nonapeptide with the amino acid sequence Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. Kallidin is a decapeptide with an identical structure to bradykinin, except an additional lysine residue is present at the amino-terminal portion; therefore kallidin is sometimes also referred to as lysyl-bradykinin (Table 28.1). Both kinins are widely distributed in nature and are known to exist in human and animals' body, certain snake venoms, and bees and wasps sting. Addition of methionine to the N-terminal lysine of kallidin yields a third biologically active kinin called methionyl-kallidin or methionyl-lysylbradykinin. In mammals, plasma kinins have been proposed as endogenous mediator of cellular responses to certain types of physiological and pathological stimuli.

Synthesis and Metabolism

Synthesis: Both bradykinin and kallidin are synthesised from the circulating $alpha_2$ globulins called kininogens by the action of specific proteolytic enzymes called kallikreins. The kininogens are synthesised in the liver and circulated in plasma in two inactive

forms – the high molecular weight (~110,000) kininogen and the low molecular weight (~70, 000) kininogen. Both types of kininogen are derived from a single gene by alternate splicing. The kallikreins, the kinin releasing proteases, also occur in two forms - plasma kallikrein and tissue/glandular kallikrein. Bradykinin is formed from high molecular weight kininogen, whereas kallidin from both high and low molecular weight kininogens by the action of plasma and tissue/glandular kallikrein enzymes, respectively.

Plasma kallikrein exists in an inactive form in the plasma called plasma pre-kallikrein, whereas tissue kallikrein occurs in an inactive form in a number of tissues including salivary gland, pancreas, kidneys, central nervous system and cardiovascular system called tissue/glandular pre-kallikrein. Plasma pre-kallikrein is activated by Hageman factor (Factor XII) which itself is activated by tissue injury, change in pH and temperature, and contact with surfaces having negative charge like collagen, basement membrane and bacterial liposaccharides (Figure 28.1). The synthesis of tissue pre-kallikrein is regulated by a number of factors including aldosterone in kidney and salivary gland, and androgens in some other glands. Activation events of tissue kallikrein are not well documented. Once kallikreins are activated, they rapidly catalyse conversion of kininogens to bradykinin and kallidin. Soon after activation, several plasma protease inhibitors inhibit kallikreins function; therefore, their capacity to form kinin autacoids is short-lived. In addition to kallikreins, kinins are generated by a number of serine proteases, trypsin and proteolytic enzymes in the snake venom and wasp venom. Bradykinin can also be generated from kallidin on the removal of lysine residue by an aminopeptidase.

Metabolism: The kinins are very rapidly metabolised ($t_{1/2}$ < 1 minute) in the body, primarily in lungs but also in other tissues by enzymes collectively called kininases. The principal kinin degrading enzyme is kininase II, which is the same peptidyl dipeptidase (angiotensin converting enzyme) that converts angiotensin I to angiotensin II. It splits off two amino acids from the carboxy terminal of the peptide chain to form inactive kinin metabolites. Kinins are also inactivated by the less specific kininase I, a carboxy peptidase that removes only one amino acid (arginine) generating selective B_1 agonistic metabolites des-Arg bradykinin and des-arg kallidin. These B_1 agonist metabolites of kinins are further degraded by other peptidases.

Mechanism of Action

Bradykinin and kallidin produce their biological effects through two types of kinin receptors – B_1 and B_2. The classical B_2 receptors selectively bind bradykinin and kallidin and are present constitutively in most tissues. The B_2 receptors mediate majority of effects of bradykinin and kallidin in the absence of inflammation. The B_1 receptor selectively binds to the des-Arg metabolites of bradykinin and kallidin and is less prevalent than the B_2 receptor. The B_1 receptors are present in normal vascular smooth muscles and are up regulated by inflammation and trauma, so that they might play a major role at inflamed sites. Bradykinin has higher affinity for B_2 than B_1 receptors, while kallidin is equipotent on both (Table 28.1).

The B_2 receptor is coupled to G-proteins and activates phospholipase C and phospholipase A_2. Kinin-induced phospholipase C activation through G_q protein leads to an increase in inositol 1,4,5-triphosphate and diacylglycerol, which in turn increase cytosolic Ca^{++} and protein kinase activity, respectively.

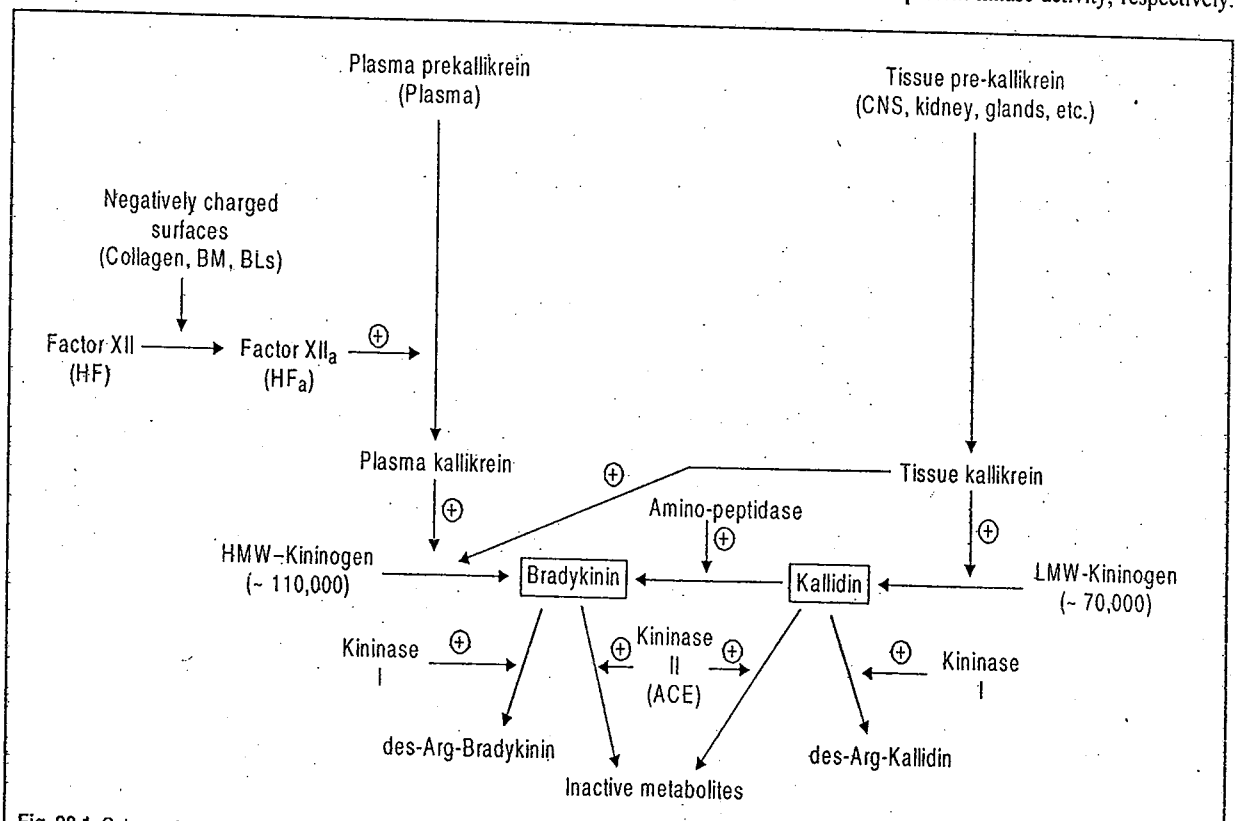


Fig. 28.1. Schematic representation of biosynthesis and metabolism of plasma kinins (see text for detail).
Abbreviations : BM = basement membrane ; BLs = bacterial liposaccharides; HF = Hageman factor ; CNS = central nervous system ; HMW = high molecular weight ; LMW = Low molecular weight ; ACE = angiotensin converting enzyme. + = activation.

Table 28.1: Chemical structures of kinins and their metabolites.

Sr. No.	Agent	Structure	Activity on kinin receptors
1.	Bradykinin	Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9	$B_2 > B_1$
2.	Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	$B_2 \cong B_1$
3.	des-Arg^9-bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	B_1
4.	des-Arg^{10}-kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	B_1

The stimulation of phospholipase A_2 through G_i protein liberates arachidonic acid from the membrane bound phospholipids, which in turn is metabolised to a number of potent inflammatory mediators (e.g. prostaglandins and leukotrienes). Binding of bradykinin to the B_2 receptor leads to internalization of the agonist–receptor complex and thus to desensitization. In contrast, the B_1 receptor does not internalize after binding its ligand, [des-Arg]-kinin. The signalling mechanism of B_1 receptor is less well characterised.

Physiological and Pharmacological Effects

Bradykinin and kallidin have similar actions and are usually taken together.

Cardiovascular system: Kinins are potent vasodilator, being about 10 times as active as histamine. However, their effect on blood vessels depends on type of vasculature, integrity of endothelium and species. In normal smooth muscles of microcirculation (terminal arterioles and small venules), kinins produce relaxation yielding a marked decrease in systemic vascular resistance and sharp fall in systolic and diastolic blood pressures. This vasodilatation is mediated either directly or indirectly through nitric oxide (NO) release. Release of histamine and other vasoactive substances from mast cells and generation of prostaglandins (e.g. PGI_2 and PGE_2) may also be involved in kinins induced vasodilatation. In contrast, large arteries, most veins and blood vessels with damaged endothelium are constricted by plasma kinins. Plasma kinins have no direct action on heart; however, a reflex increase in heart rate and cardiac output may occur due to fall in blood pressure. The kallikrein-kinin system appears to be cardioprotective. It contributes to the beneficial effect by preconditioning of the heart against ischemia reperfusion injury, prevents vascular smooth muscle growth and cell proliferation and stimulates tissue plasminogen activator (tPA).

Inflammation: Kinins play an important role in the inflammatory process and produce clinical signs of inflammation such as redness, local heat, swelling and pain. Intradermal injection of kinins produces the classic triple response as seen with histamine. Plasma kinins increase permeability in the microcirculation by widening the intercellular spaces between the endothelial cells leading to exudation and oedema formation. Stimulation of B_1 receptors on inflammatory cells such as macrophages can elicit production of inflammatory mediators interleukin 1 and tumour necrosis factor alpha.

Pain: Kinins are powerful algesic agents, when applied topically or injected intradermally. They evoke pain and itching by direct stimulation of nociceptive nerve endings in the skin and viscera.

Smooth muscles: Bradykinin and kallidin contract most of non-vascular smooth muscles. Bronchoconstriction by kinins is prominent in some species (e.g. guinea pigs) and asthmatic patients. Kinins induced contraction of intestine is slow (bradykinin owes its name to its ability to induce slow contractions in intestinal smooth muscles).

Kidneys: Kinins regulate urine volume and composition. They increase renal blood flow and facilitate salt and water excretion by enhancing the electrogenic transport of chloride in the collecting duct.

Exocrine and Endocrine glands: Kinins may modulate the tone of ducts of various glands such as salivary and pancreatic glands and help regulate gastrointestinal motility. They are known to stimulate ion-transport and fluid secretion in various epithelia including intestine, airways and gall bladder. Kinins influence the physiological activation of various prohormones including proinsulin and prorenin. Kinins also function in the male reproductive system in areas such as spermatogenesis and in promoting sperm motility.

Other effects: Kinins can stimulate autonomic ganglia and release catecholamines from the adrenal medulla. The kinins also have neurochemical effects in the CNS. Kinins play a role in transition from foetal to neonatal circulation e.g. dilation of foetal pulmonary artery, closure of ductus arteriosus and constriction of umbilical vessels.

Patho-physiological Roles

1. *Regulation of microcirculation:* The release of kinins and tissue kallikrein may be important in controlling the blood flow to certain exocrine glands and thus controlling glandular secretions. They have also been implicated in reactive hyperaemia, regulation of tissue blood flow and blood pressure control. An important relationship seems to exist between kinins (potent vasodilators) and angiotensin (potent vasoconstrictor) because the activating enzyme (angiotensin converting enzyme or kininase II) in the angiotensin system is a strong inactivator of kinins.
2. *Inflammation:* Kinins participate in a variety of inflammatory diseases. Tissue injury can cause local kinin production, which then sets in motion the inflammatory defensive and reparative processes. Increased kinin levels have been observed in a number of inflammatory conditions e.g. rhinitis, gout, disseminated intravascular coagulation and inflammatory bowel disease.
3. *Allergic reactions and shock:* Kinins are involved in many pathological states like allergy, shock and bronchoconstriction. Kinins may also play important roles in conditions like rhinitis, angiodema, asthma and carcinoid syndrome.
4. *Pain:* The kinins are powerful algesic agents which act as mediators of pain by directly stimulating nerve endings and by increasing prostaglandin production. They also provoke release of neuropeptides such as substance P, neurokinin A and calcitonin-gene-related peptide (CGRP).

5. ***Blood coagulation:*** Production of kinins is integrated with clotting, fibrinolysis and complement systems. Liberated kallikrein activates Factor XII by a positive feedback mechanism, which is independent of kinin production.
6. ***Other roles:*** Excessive bradykinin production is probably a factor in causing fluid secretion in diarrhoea in many GI disorders. Acute pancreatitis and certain immunological reactions have been associated with kinin production.

PHARMACOLOGY OF KININS

The bradykinin and kallidin have no therapeutic use, but a few drugs which modify the activity of kallikrein-kinin system are either available for use in clinical practice or undergoing clinical trials.

Drugs Enhancing Activity of Kallikrein-kinin System

RMR-7

RMP-7 [H-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-4 Me-Tyr(ψCH_2NH)-Arg-OH] is a bradykinin analogue that is resistant to degradation by kinin metabolising enzymes. It increases permeability of blood-brain barrier to certain drugs. Presently the drug is being evaluated for its efficacy in increasing the delivery of chemotherapeutic agents into the CNS for treatment of primary brain tumours.

Angiotensin converting enzyme (ACE) inhibitors

Angiotensin converting enzyme (ACE) inhibitors, also known as kininase II inhibitors, are the drugs which block angiotensin converting enzyme and prevent formation of angiotensin II from angiotensin I. These drugs also prevent the degradation of endogenous bradykinin and kallidin and prolong their duration of action. Although the ACE-inhibitors are clinically not used to prolong the actions of kinins, but some of their antihypertensive effects are mediated through vasodilatory action of bradykinin and kallidin.

Drugs Inhibiting Acitivity of Kallikrein-kinin System

1. Kinins Synthesis Inhibitors

Aprotinin

Aprotinin is a polypeptide obtained from bovine lungs. It has a molecular weight of 6512. It inhibits kallikreins and hence blocks synthesis of kinins. Aprotinin also inhibits several serine proteases, specifically trypsin, chymotrypsin and plasmin. Its action on kallikrein leads to the inhibition of the formation of factor XIIa. As a result, both the intrinsic pathway of coagulation and fibrinolysis are inhibited. Its action on plasmin independently slows fibrinolysis.

Aprotinin is used in human patients at high risk of blood loss during and after open heart surgery and life-threatening haemorrhage due to hyperplasminaemia. Its main purpose is to reduce the bleeding by slowing down fibrinolysis and hence requirement of blood transfusion during complex surgery. Hypersensitivity reactions including anaphylactic or anaphylactoid reactions is the main concern with aprotinin. Thrombosis, presumably from overactive inhibition of the fibrinolytic system, may also occur at a higher rate. Presently the drug has been temporarily withdrawn from market due to enhanced adverse effects.

2. Kinin Receptor Antagonists

Icatibant

Icatibant (HOE 140) is a recently developed peptidomimetic drug consisting of ten amino acids. It selectively binds with and antagonises kinin B_2 receptor. As a result, the endogenous bradykinin fails to bind to the bradykinin B_2 receptor. It has been approved for use in humans for the symptomatic treatment of acute attacks of hereditary angioedema (HAE), a conditions characterised by swelling and oedema of the head and neck regions. Icatibant has received status of orphan drug due to its approval for use in rare diseases.

des-Arg-HOE 140

des-Arg-HOE140 is a selective antagonist for B_1 receptor. It is available for research use only.

ANGIOTENSINS

Angiotensins are a family of peptide autacoids which are involved in electrolyte, blood volume and blood pressure homeostasis. Although three main types of angiotensins i.e. angiotensin I, angiotensin II and angiotensin III are present in blood, the angiotensin II is most active biologically and is usually called the angiotensin.

Chemistry

Angiotensins are blood-borne polypeptides which are formed within the blood by a complex series of reactions initiated by renal enzyme renin. Angiotensin II is an octapeptide, whereas angiotensin I and angiotensin III are decapeptide and heptapeptide, respectively (Figure 28.2). They form an integral part of the better known "renin-angiotensin system and primarily serve as a circulating link between kidney and systemic haemodynamic control systems in the body.

Biosynthesis and Metabolism

Angiotensins are synthesised from the precursor of plasma α_2-globulin, the angiotensinogen. Angiotensinogen is produced constitutively and released into the circulation mainly by the liver. Renin, a proteolytic enzyme secreted by juxtaglomerular cells in the kidney separates a decapeptide from angiotensinogen to form angiotensin I. Release of renin is stimulated by ischemia, hypovolaemia, hypotension, hyponatraemia and β-adrenoceptor activation. Angiotensin I appears to have no biological activity and exists solely as a precursor to angiotensin II. Angiotensin I is acted upon by angiotensin converting enzyme (ACE), a peptidyl dipeptidase enzyme found in large amounts in lung capillary endothelial cells and other vascular beds, and metabolises it to the biologically active octapeptide angiotensin II. Subsequently, angiotensin II ($t_{½}$ = 1 min) is metabolised by an aminopeptidase to angiotensin III, which retains some biological activity. Angiotensin III is further acted upon by a variety of peptidases, collectively called angiotensinases, to inactive fragments.

Renin and Regulation of Renin-Angiotensin System

Renin is an aspartyl protease that attacks a restricted number of substrates, of which the major one is a circulating alpha$_2$-globulin angiotensinogen. Renin is synthesized as a preproenzyme of 406 amino acids that is processed to prorenin, an inactive form

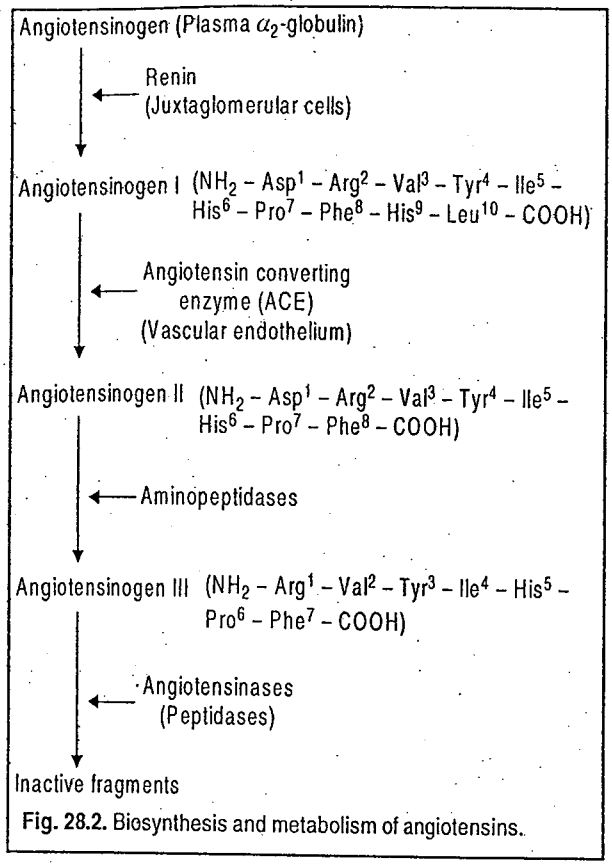


Fig. 28.2. Biosynthesis and metabolism of angiotensins.

of the protein which subsequently forms the active renin containing 340 amino acids. Both renin and prorenin are synthesized and stored in juxtaglomerular cells and on release enter the circulation. The secretion of renin from juxtaglomerular cells acts as the primary regulating factor for angiotensin synthesis and secretion. In general, factors which reduce blood volume, renal perfusion pressure or plasma sodium concentration tend to stimulate release of renin through the intrarenal baroreceptor mechanism afferent arteriols, intrarenal chemoreceptor mechanism of macula densa, renal sympathetic nerves and several humoral factors (Figure 28.3). In contrast, renin secretion is inhibited by increased blood volume or blood pressure. Vasopressin, angiotensin II and plasma K^+ also decrease renin secretion. The half life of renin is about 15 minutes.

Local/Tissue Renin-Angiotensin System

Recent evidence suggests that in addition to circulating angiotensins, several organs can synthesise angiotensin II locally in the tissues. Blood vessels can capture circulating renin and angiotensinogen and produce angiotensin II within their walls. Many tissues including brain, pituitary, blood vessel, heart, kidney and adrenal gland express mRNAs for renin, angiotensinogen and/or ACE and appear to produce angiotensins locally. The physiological significance of these tissue renin-angiotensin systems is still not clear because no single cell type within a given tissue appears to express all components of the system. The local systems do not contribute significantly to circulating levels of active renin or angiotensins.

Mechanism of Action

The effects of angiotensins are exerted through two types of G protein-coupled receptors - AT_1 and AT_2. The AT_1 and AT_2 receptors share a sequence identity of less than 30%, but have a similar affinity for angiotensin II, which is their main ligand.

AT_1 receptors: All pharmacological effects of angiotensin II appear to be mediated by the AT_1 receptors. These are coupled to $G_{q/11}$ protein, stimulation of which leads to activation of phospholipase C. The phospholipase C, in turn, generates inositol triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidyl inositol causing release of Ca^{++} from the endoplasmic reticulum and activation of protein kinase, respectively (see for detail Chapter 6). Both events contribute to contraction of smooth muscle. The AT_1 receptor activation also leads to stimulation of phospholipase A_2 to form arachidonic acid that in turn is metabolised to prostaglandins, leukotrienes and thromboxanes. In some cell types, AT_1 receptor activation decreases intracellular cAMP by inhibiting adenylate cyclase enzyme.

AT_2 receptors: The AT_2 receptors are widely distributed in foetal and neonatal tissues, but appear to have limited distribution in adults. The AT_2 receptor has no known functional role. They have little sequence homology with AT_1 receptors. Signalling from AT_2 receptors is mediated largely by G_i proteins. This results in activation of phosphatases, potassium channels, bradykinin and nitric oxide production and inhibition of calcium channel functions. The AT_2 receptor largely remains enigmatic and controversial.

Physiological and Pharmacological Effects

Angiotensin II is the biologically most active angiotensin having significant effects on blood vessels, heart, kidneys and adrenal medulla. Angiotensin II and III stimulate aldosterone equally, but the potency of angiotensin III is only 25% and 10% as that of angiotensin II in elevating the blood pressure and stimulating the adrenal medulla respectively. The potency of angiotensin I is only 1% as that of angiotensin II on smooth muscle, heart and adrenal cortex.

Blood vessels: The renin angiotensin system is involved in short (rapid) term and long (slow) term maintenance of blood pressure in the body. Angiotensin II is one of the most potent vasoconstrictors known, being 40 times more potent than norepinephrine. Vasoconstriction involves arterioles and to lesser extent venules, and occurs in all vascular beds. Vasoconstriction is marked in kidney, skin and splanchnic tissues, but less significant in cerebral, skeletal muscle, pulmonary and coronary vessels. The vasoconstrictor effect is produced by direct action on AT_1 receptor located in vascular smooth muscle cells as well as by enhancing release of norepinephrine/epinephrine from adrenal medulla/adrenergic nerve endings and by increasing central sympathetic outflow. Net result of vasoconstriction is increase in peripheral vascular resistance and both diastolic and systolic blood pressures.

Heart: Angiotensin II has positive inotropic and chronotropic effects due to central and peripheral sympathetic stimulation. However, reflex bradycardia may occur in intact animal. Cardiac output is often decreased.

Cellular growth: Angiotensin II acting on a chronic basis induces hypertrophy, hyperplasia and increased intracellular matrix production in the myocardium and vascular smooth muscle. These effects are mediated in part by direct cellular effects involving

expression of proto-oncogens and transcription of several growth factors. Indirectly, volume overload, increased peripheral resistance and long-standing hypertension caused by angiotensin II contribute to the vascular and ventricular hypertrophy.

Blood coagulation: Angiotensin II has prothrombotic potential through adhesion and aggregation of platelets and production of platelet activator inhibitors.

Adrenal cortex: Angiotensin II (also angiotensin III) promotes the synthesis and secretion of aldosterone by the adrenal cortex *zona glomerulosa*. Aldosterone facilitates sodium and water retention and potassium loss (see for detail Chapter 38). These effects are exerted at concentrations lower than those required to cause vasoconstriction.

Kidneys: Angiotensin II increases Na^+ and water reabsorption both directly by reducing renal blood flow and indirectly through increased aldosterone and antidiuretic hormone. However, an opposite effect has been observed in cirrhotics and renovascular disease patients.

Sympathetic nervous system: Angiotensin II enhances sympathetic nervous system activity by facilitating release of norepinephrine from post-junctional sympathetic neurons, stimulating sympathetic ganglia, decreasing reuptake of norepinephrine into adrenergic axon, and provoking release of norepinephrine and epinephrine from adrenal medulla. Angiotensin also increases central sympathetic outflow, which contributes to enhanced sympathetic activity and pressor response.

Other effects: Angiotensin II contracts many visceral smooth muscles *in vitro*, but *in vivo* effects are insignificant. Angiotensin II through its central effect enhances secretions of ACTH and vasopressin and stimulates drinking (dipsogenic effect).

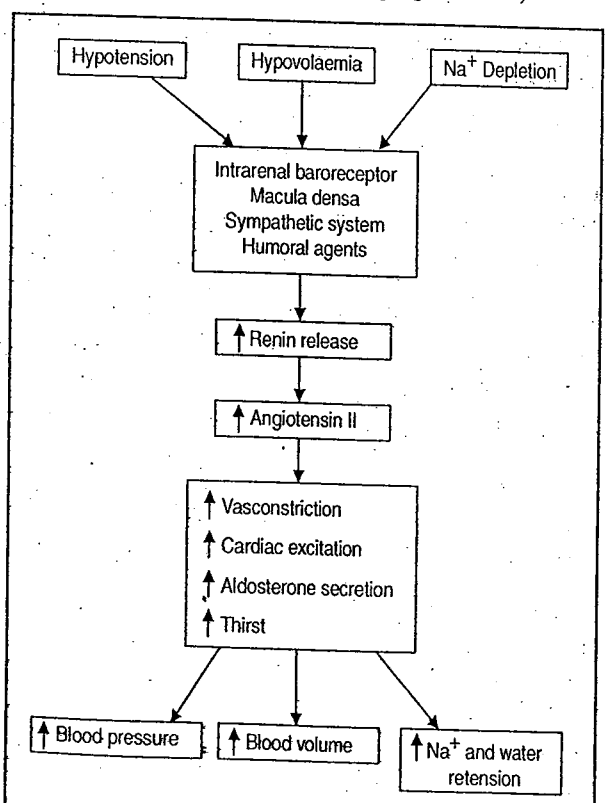


Fig. 28.3. Schematic representation of physiological regulation of electrolyte, blood pressure and blood volume by the renin-angiotensin system.

Patho-physiological Roles

1. ***Blood pressure homeostasis:*** The renin-angiotensin system plays an important role in maintaining the blood pressure. In acute hypotensive states (e.g. blood loss and vasodilatation), angiotensin causes a swift increase in total peripheral resistance through a number of mechanisms (see effect on blood vessels) and helps maintain arterial blood pressure (often called rapid pressure response). Angiotensin II also causes a slow pressor response thorough a decrement in renal excretory function that helps stabilise arterial blood pressure over long-term.
2. ***Electrolyte and blood volume:*** The renin-angiotensin system increases Na^+ and water reabsorption in kidneys and plays a significant role in maintaining electrolyte composition and volume of extracellular fluid.
3. ***Mineralocorticoid secretion:*** Angiotensins II (also angiotensin III) is the physiological stimulus for aldosterone secretion from adrenal cortex. It also exerts trophic influence on the zona-glomerulosa cells of the adrenal cortex. Renin-angiotensin system is also involved in development of secondary hyperaldosteronism.
4. ***Cardiovascular diseases:*** The renin-angiotensin system is directly involved in the reno-vascular hypertension. Inhibition of renin-angiotensin system lowers systemic vascular resistance and blood pressure in various hypertensive states (see for detail Chapter 34). The role of angiotensin II has also been clearly implicated in development of atherosclerosis, congestive heart failure, left ventricular dysfunction following infarction and diabetic nephropathy.
5. ***Central nervous system:*** It has been established now that angiotensin II can be formed within the CNS and may function as neurotransmitter or neuromodulator. Regulation of thirst, release of some hormones (e.g. ADH and ACTH) and sympathetic outflow may be the responses mediated by angiotensins.

PHARMACOLOGY OF ANGIOTENSINS

ANGIOTENSIN AND ANALOGUES

Angiotensin

Angiotensin II is not commercially marketed as a drug and is not approved for veterinary use. Rarely, angiotensin II has been used clinically in human medicine to counter hypotension during anaesthesia with agents which sensitise heart to adrenergic arrhythmia. However, it often precipitates myocardiac ischemia.

Angiotensin II Amide

Angiotensin II amide is a synthetic compound that produces sustained rise in blood pressure when given by intravenous infusion. However, it may cause vasoconstriction that may decrease tissue perfusion, especially when used in the treatment of refractory shock. Like angiotensin, it has limited therapeutic utility.

INHIBITORS OF ANGIOTENSIN OR RENIN-ANGIOTENSIN SYSTEM

Inhibition or antagonism of angiotensin or renin-angiotensin system can be achieved by use of different groups of drugs. Some of these drugs are clinically important.

Classification

On the basis of mechanism of action and chemical structures, inhibitors of renin-angiotensin system are divided into following four groups:

I. Renin-secretion inhibitors
   e.g. propranolol.
II. Renin inhibitors
   e.g. aliskiren and remikiren.
III. Angiotensin converting enzyme (ACE) inhibitors
   1. Sulphydryl containing ACE inhibitors
      e.g. captopril and zofenopril.
   2. Dicarboxyl containing ACE inhibitors
      e.g. enalapril, benazepril, lisinopril, ramipril, imidapril, quinapril, perindopril and trandolapril.
   3. Phosphinate containing ACE inhibitors
      e.g. fosinopril.
IV. Angiotensin receptor antagonists
   1. Peptide antagonists
      e.g. saralasin.
   2. Non-peptide antagonists
      e.g. losartan, irbesartan, candesartan, eprosartan, valsartan, olmesartan and telmisartan.

I. Renin-Secretion Inhibitors

Renin-secretion inhibitors are primarily antagonists of sympathetic nervous system. It is known that activation of β_1-adrenoceptors on juxtaglomerular cells of kidney enhances renin secretion; therefore, blockade of renal sympathetic activity by β-adrenoceptor antagonists (e.g. propranolol) decreases renin secretion. Decrease in renin secretion in turn decreases the formation of angiotensin II. This is one of the mechanisms, which explains the hypotensive action of β-adrenoceptors antagonists such as propranolol.

II. Renin-Inhibitors

Various types of renin inhibitors have been developed recently, which block the action of renin and interfere with generation of angiotensin I from angiotensinogen. These include renin antibodies, analogues of pro-renin and inhibitors of renin. All these groups of drugs, except inhibitors of renin, did not find much use in therapeutics because of their low bioavailability and short duration of action. Inhibitors of renin (e.g. aliskiren and remikiren) produce a dose-dependent reduction in plasma renin activity and are used occasionally for reduction of elevated blood pressure.

Aliskiren

Aliskiren belongs to a new class of non-peptide drugs which inhibit activity of plasma renin and produce a dose dependent fall in blood pressure. It is an octanamide, low-molecular weight and orally active drug. Aliskiren due to its structural composition binds to an active site on renin, which is essentially required for its activity. As a result of this, the angiotensinogen cannot occupy the active site on renin and thus there is no conversion of angiotensinogen to angiotensin I. This results in reduction in plasma renin activity and hence decrease in angiotensin I, II and aldosterone concentration. The pharmacological effects of aliskiren and other renin-inhibitors may be limited because when these drugs are used chronically, the continuous interruption of negative feedback mechanisms often increases renin production.

Aliskiren is designed by the use of molecular modelling techniques and is mainly used in the treatment of hypertension in human beings. Aliskiren has good water solubility and low lipophilicity and is resistant to biodegradation by peptidases in the intestine, blood circulation and liver. It has a plasma half-life of about 24 hours in man. Angioedema, hypotension, diarrhoea and other GI symptoms, dizziness and headache are important side effects in human beings. Use of aliskiren along with ACE-inhibitors is associated with hyperkalemia and foetal malformation. Aliskiren is currently are not available for veterinary use.

Remikiren

Remikiren is another renin inhibitor with action similar to that of aliskiren. Currently, it is under development for the treatment of hypertension.

III. Angiotensin Converting Enzyme Inhibitors

Angiotensin converting enzyme (ACE) inhibitors are drugs that block angiotensin converting enzyme and prevent formation of angiotensin II from angiotensin I. These drugs are widely used in both human and veterinary medicines and many ACE inhibitors are available commercially. In veterinary practice, ACE inhibitors are used mainly in the treatment of chronic congestive heart failure in dogs, where they have shown to prolong life and improve the quality of life in most dogs. ACE inhibitors have also been used to improve ventricular diastolic function in cardiomyopathies, especially in cats, probably by inhibiting the remodelling effects of angiotensin II.

1. Sulphydryl Containing ACE-Inhibitors

Captopril

Captopril is a sulphydryl containing dipeptide surrogate of proline and was the first ACE inhibitor to be marketed. The development of captopril in 1975 was considered a breakthrough both because of its novel mechanism of action and also because of the revolutionary structure-based drug development process. Captopril is freely soluble in water and alcohol and occurs as white to off-white crystalline powder. It is considered as prototype drug because its effects are common to all ACE inhibitors.

Mechanism of action

Captopril is a potent inhibitor of angiotensin converting enzyme. It has much higher affinity for the active site of ACE than the angiotensin I. Therefore, it competes with angiotensin I for the ACE and binds to the enzyme site normally occupied by the angiotensin I. This blocks the formation of angiotensin II. Captopril also diminishes the rate of inactivation of bradykinin because kinins are normally metabolised by ACE (also called kininase II). By reducing circulating angiotensin II levels, captopril and other ACE inhibitors also decrease the secretion of aldosterone resulting in decreased sodium and water retention. Unlike most ACE inhibitors, captopril is not a pro-drug.

Pharmacological effects

Captopril reduces total peripheral resistance and lowers both systolic and diastolic blood pressures. These effects are produced as a result of the combined effects of lower vasoconstriction

caused by decreased levels of angiotensin II and vasodilatation effect of increased bradykinin. Cardiovascular reflexes are not interfered with and there is little dilation of capacitance vessels. It can induce positive haemodynamic effects within 1 hour, although the effects are short lived (~ 4 hours). Captopril and other ACE inhibitors do not affect cardiac contractility, rate or output. The renal, cardiac and cerebral blood flow is not compromised even when blood pressure falls substantially. Levels of plasma renin and angiotensin are increased as compensatory measure because captopril interferes with negative feedback mechanism on renin release. The physiological significance of this appears to be minor.

Captopril also reduces aldosterone secretion as a consequence of loss of its regulation by angiotensin II. As a result, natriuresis (loss of sodium in urine) may occur.

Pharmacokinetics

After oral administration, captopril is rapidly absorbed and has a bioavailability of about 75% in dogs. However, presence of food in stomach reduces its bioavailability. It binds to plasma proteins (up to 40%) and distributes to most tissues. Penetration of captopril in the brain is poor. Unlike many ACE inhibitors, captopril does not require conversion in the liver to its active metabolite. It is rapidly metabolised in liver and excreted in urine, both as unchanged drug and as metabolites. Its half-life is short (<3 hours in dogs) and as such requires repetition at 8 hour intervals.

Side effects/Adverse effects

Important side/adverse effects related to captopril include hypotension, hyperkalaemia, GI disturbances (anorexia, vomiting and diarrhoea) and renal dysfunction. Unlike in human beings, rashes, urticaria and neutropenia/agranulocytosis are not commonly seen in dogs. Transient and mild azotemia may develop and monitoring of BUN and creatinine may be required.

Treatment: Captopril overdosage should be treated symptomatically and supportively. Volume expansion with normal saline is recommended.

Contraindications and precautions

Captopril is contraindicated in animals at risk of hypotension and patients hypersensitive to ACE-inhibitors. Captopril and other ACE inhibitors are generally not recommended in pregnant animals as they cross placenta and may cause foetal malformation and death. Captopril should be cautiously used in hepatic or renal insufficiency. Patients with severe congestive heart failure should be monitored carefully. Renal function should be monitored before and during captopril therapy. As food reduces its bioavailability, captopril should be given 1 hour before meal.

Drug interactions

Concomitant use of diuretics, other vasodilators and NSAIDs attenuates the hypotensive effect of captopril. Concomitant use of potassium or potassium sparing diuretics (e.g. spironolactone) may cause hyperkalaemia. Antacids reduce bioavailability of captopril.

Clinical uses

In veterinary medicine, captopril is used mainly as a vasodilator in the treatment of congestive heart failure (CHF) and hypertension. It is primarily used in dogs, but may be considered in cats. ACE inhibitors are not considered a primary treatment for hypertension in cats. Captopril has also been shown to have a protective effect in some cases of progressive renal disease.

Dose

Dogs : 0.25 – 2 mg/kg, PO, 3 times daily.

Cats : 3 – 6 mg (total), PO, 2 times daily.

Other sulphydryl containing ACE-inhibitors

Other sulphydryl containing ACE-inhibitors include zofenopril, pivalopril and alaceperil. These drugs are less commonly used in therapeutics.

2. Dicarboxyl-Containing ACE-Inhibitors

Enalapril

Enalapril was the first member of the group of ACE inhibitors known as the dicarboxylate-containing ACE. It was developed primarily to overcome the side effects of captopril. Enalapril is widely used in veterinary and human medicines.

Chemistry and mechanism of action

Enalapril is structurally related to captopril but unlike captopril it does not possess a sulphydryl group. Unlike captopril, enalapril is not active itself, but it forms an active molecule enalaprilat *in vivo*. Enalaprilat is a potent inhibitor of ACE, which prevents formation of angiotensin II from angiotensin I and reduces secretion of aldosterone.

Pharmacological effects

The pharmacological effects of enalapril/enalaprilat are related to inhibition of ACE and are same of captopril. In patients with congestive heart failure, there is reduced total peripheral resistance, hypotension, no change or decrease in heart rate and increased cardiac output. Some venodilation reduces pre-load as well, which may be of benefit. Renal blood flow can be measured with little change in hepatic blood flow.

Pharmacokinetics

Enalapril is rapidly absorbed when given orally. Unlike captopril the absorption of enalapril in not much influenced by presence of food in the GI tract. After absorption, enalapril is hydrolysed by esterases in liver to produce the active compound enalaprilat (Figure 28.4). Therefore, it has a delayed onset (4 to 6 hours) and a long duration (12 to 24 hours) of action. Both enalapril and enalaprilat distributes poorly into CNS but crosses placenta and also distributes in milk in trace amounts. The half-life of enalapril/enalaprilat is increased in animals with severe congestive heart failure or renal insufficiency.

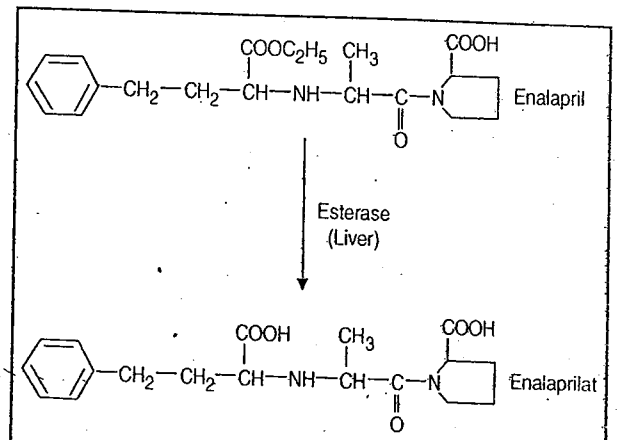


Fig. 28.4. Biotransformation of enalapril to active metabolite enalaprilat.

Side effects/Adverse effects

Enalapril has the same adverse effect profile as captopril, but it is reported to have lesser incidence of GI side effects. Potentially hypotension, renal dysfunction and hyperkalaemia could occur. Enalapril may cause transient and mild azotaemia, lethargy, drowsiness, hypotension and incoordination in animals.

Contraindications and precautions

Enalapril should not be used in animals at risk of hypotension, cardiac failure or renal insufficiency. Renal impairment results in significant accumulation of enalaprilat that necessitates dosage reduction. Safety of enalapril in pregnant animals has not been evaluated, so it should not be used in pregnant bitches. Therapy with enalapril and other ACE inhibitors should be closely monitored.

Drug interactions

Drug interactions of enalapril are similar to those of captopril.

Clinical uses

Enalapril maleate is one of the most widely used ACE inhibitor in veterinary medicine. It is indicated mainly in the treatment of CHF in dogs and cats and also in the treatment of hypertension. It is considered useful in treating the effects associated with valvular heart disease and left to right shunts. Treatment with enalapril is usually initiated at a lower dose rate and increased in small increments at weekly intervals until clinical signs indicate improvement or further increase in dose cannot be tolerated. Enalapril is available in 1, 2.5, 5, 10 and 20 mg tablets for veterinary use.

Dose

Dogs : 0.5 mg/kg, PO, once daily. Dose may be increased to twice daily, if required.

Cats : 0.25 – 0.5 mg/kg, PO, 1 to 2 times daily.

Enalaprilat

Enalaprilat is an active metabolite of enalapril, which is also marketed as a drug. It is a potent inhibitor of ACE, but is not absorbed orally. It is mainly used by IV route when oral therapy is not appropriate. Enalaprilat is injected directly into a vein (IV) over 5 minutes or is mixed in a dextrose-water (e.g. D5W) or saline solution (NS) usually every 6 hours. The use of enalaprilat in veterinary medicine is limited.

Benazepril

Benazepril HCl is a non-sulphydryl ACE inhibitor. Like enalapril, benazepril is a pro-drug, which is hydrolysed in the liver to an active compound benazeprilat. Benazeprilat is a potent inhibitor of ACE; it inhibits plasma ACE for more than 24 hours after a single dose. Benazepril after oral dosing is rapidly absorbed. Peak concentrations of benazepril and benazeprilat in plasma are achieved in about 0.5 to 1 hour and 1 to 1.5 hours respectively. The elimination half-life of benazeprilat is approximately 3.5 hours in dogs. About half of benazeprilat undergoes hepatic excretion. Benazepril has the same pharmacological effects, adverse effects and therapeutic profile as captopril and enalapril. It should not be used in animals at risk of hypotension and in pregnant or lactating animals, unless the benefit-risk ratio is considered justified. Veterinary preparations of benazepril HCl are available for treatment of congestive heart failure and hypertension in dogs and chronic renal failure in dogs and cats.

Dose

Dogs : 0.25 – 0.5 mg/kg, PO, once daily.

Cats : 0.5 – 1 mg/kg, PO, once daily.

Lisinopril

Lisinopril is the lysine analogue of enalapril. However, unlike enalapril and many other ACE inhibitors, lisinopril itself is active i.e. it does not require hydrolysis to become active ACE inhibitor. Lisinopril has some other properties which distinguish it from other ACE inhibitors: it is hydrophilic, has long half-life and tissue penetration, and is not metabolized in liver. Its oral absorption is slow and incomplete, but unaffected by food. After absorption, it does not undergo metabolism and is excreted largely unchanged in the urine. Its indications, contraindications and side effects are as those for enalapril and other ACE inhibitors. Its long half-life allows for once a day dosing.

Dose

Dogs & cats : 0.25 – 0.5 mg/kg, PO, once daily.

Ramipril

Ramipril is another angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure in human beings and dogs. Ramipril is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes. Ramiprilat is mostly excreted by the kidneys. The half-life of ramiprilat is variable (3–16 hours) and is prolonged by heart and liver failure, as well as kidney failure. Hypotension, fatigue, lethargy, ataxia are important side/adverse effects observed in small animals.

Dose

Dogs : 0.12 mg/kg, PO, once daily. Dose may be increased to 0.25 mg/kg, once daily after 2 weeks.

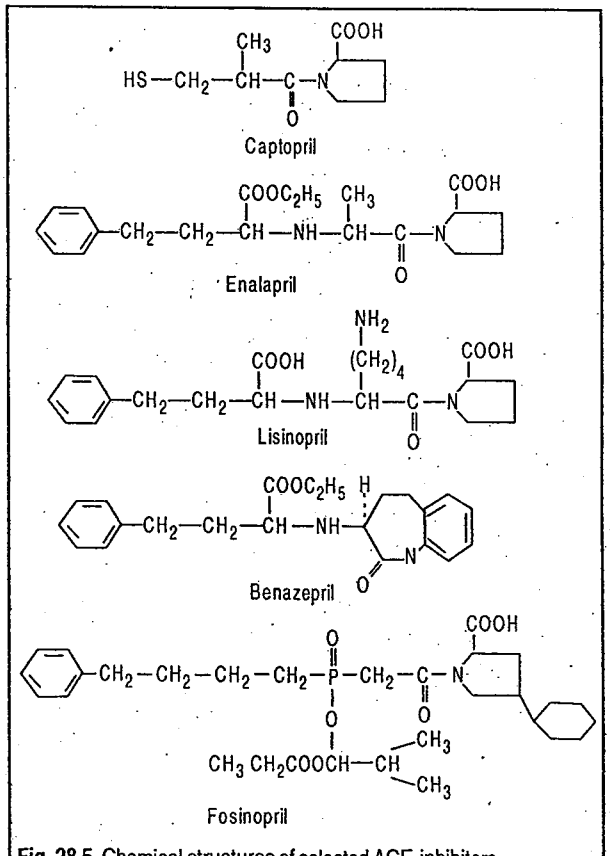


Fig. 28.5. Chemical structures of selected ACE-inhibitors.

Table 28.2: Comparative features of selected ACE-inhibitors in dogs.

Feature	Captopril	Enalapril	Benazepril	Lisinopril	Fosinopril
1. Subtype containing	Sulphydryl containing	Dicarboxyl containing	Dicarboxyl containing	Dicarboxyl containing	Phosphinate
2. Nature of parent drug	Active	Pro-drug	Pro-drug	Active	Pro-drug
3. Active metabolite	Nil	Enalaprilat	Benazeprilat	Nil	Fosinoprilat
4. Duration of action	4-6 hours	12-24 hours	24 hours	24 hours	–
5. Mode of excretion	Renal	Renal	Renal and hepatic	Renal	Renal and hepatic

Imidapril

Imidapril is a recently introduced long acting prodrug ACE inhibitor. In veterinary medicine, it has been recommended for the treatment of hypertension and moderate to severe heart failure due to mitral valve regurgitation or dilated cardiomyopathy. Important side effects in dogs are hypotension, anorexia, diarrhoea and lethargy. Similar to other ACE-inhibitors, imidapril is contraindicated in pregnant and lactating bitches, existing hypotension and acute renal impairment.

Dose

Dogs : 0.25 mg/kg, PO, once daily.

Other drugs

Several other dicarboxyl containing ACE-inhibitors are available commercially, but are less used in veterinary medicine. These include cilazapril, indolapril, moexipril, perindopril, pentopril, quinapril, spirapril and trandolapril. All are pro-drugs which are relatively inactive until converted to their corresponding di-acids.

3. Phosphinate-Containing ACE-Inhibitors

Fosinopril

Fosinopril sodium contains a phosphinate group and is the only important drug of its subclass. Like many other ACE-inhibitors, fosinopril is a pro-drug that must be hydrolysed by esterases in liver to produce the active compound fosinoprilat. It is glucuronide conjugated and excreted by both liver and kidneys. Fosinopril is approved for human use only for the treatment of hypertension and some types of chronic heart failure.

IV. Angiotensin Receptor Antagonists

1. Peptide Antagonists

Saralasin

Saralasin is an octapeptide analogue of angiotensin II (bovine) with amino acids 1 and 8 replaced with sarcosine and alanine, respectively. It is a highly specific competitive inhibitor of angiotensin II. It competitively blocks both AT_1 and AT_2 receptors and inhibits vasoconstrictor and aldosterone releasing effects of angiotensin II. Saralasin is not used clinically nowadays, because it is reported to have partial agonistic activity and in absence of high angiotensin II levels it may paradoxically increase the blood pressure. It also has very poor bioavailability, which requires IV administration.

2. Non-Peptide Antagonists

Non-peptide orally active angiotensin II receptor antagonists (ARB) are now available. They bind to the AT_1 receptor with a high affinity and inhibit most of the biological effects of angiotensin II on the vascular smooth muscle, kidney, adrenal cortex sympathetic neurotransmission, cellular hypertrophy and hyperplasia. They differ from ACE inhibitors in that they reduce activation of AT_1 receptors more effectively, divert angiotensin II to activate AT_2 receptors and do not increase levels of bradykinin. Important AT_1 receptor antagonists include losartan and irbesartan.

Losartan

Losartan is a newly introduced non-peptide competitive blocker of AT_1 receptor. It is devoid of partial agonistic activity and is reported to be about 10,000 times more selective for AT_1 than AT_2 receptors. It is a competitive antagonist, but the inhibition of biological response to angiotensin II may be insurmountable. It blocks angiotensin II induced vasoconstriction, central and peripheral sympathetic stimulation, release of aldosterone and secretion of catecholamines from adrenal medulla. No effect on angiotensin converting enzyme or bradykinin level has been reported. In human beings, the antihypertensive potency of losartan is reported to be equal to that of enalapril.

Losartan is well absorbed following oral administration in human beings and undergoes significant first-pass metabolism to produce an active 5-carboxylic acid metabolite, designated as EXP3174. This metabolite is long-acting (6 to 8 hr) and 10-40 times more potent in blocking AT_1 receptors than the parent drug losartan. It contributes significantly to the pharmacological effects of losartan and its antagonism appears to be non-competitive at the AT_1 receptor. It is reported that dogs do not convert losartan to the active metabolite. Accordingly, the losartan has weaker AT_1-receptor inhibition and has shorter duration of action in dogs. At present, losartan is approved only for human beings for treatment of hypertension (see also Chapter 34). Similar to ACE-inhibitors, hypotension and hyperkalaemia are important side effects of losartan.

Irbesartan

Irbesartan is an angiotensin II receptor antagonist with pharmacological effects similar to those of losartan. It is mainly recommended in human medicine as an alternative to ACE inhibitors for the treatment of hypertension. Irbesartan has also been used in dogs and has shown to block angiotensin II effects. No adverse effects of irbesartan have been reported in animals, although hypotension is a potential problem from overdosing. Irbesartan should not be recommended in dehydrated or hypotensive patients.

Dose

For hypertension

Dogs : 30 – 60 mg/kg, PO, 2 times daily.

Other drugs

Several other AT_1 receptor antagonists are available for therapeutic use in human medicine. These include candesartan, eprosartan, olmesartan, telmisartan valsartan and zolasartan. Selective antagonists of AT_2 receptors as well have been produced. Because no role for AT_2 receptors has yet been discovered, selective AT_2 and non-selective (AT_1 and AT_2) angiotensin receptor antagonists are not of clinical significance.

TACHYKININS

Tachykinins are a family of peptides widely expressed in the central nervous system and peripheral tissues of vertebrates and invertebrates. These include three physiologically and patho-physiologically important autacoid peptides viz. substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). These tachykinins are involved in excitation of neurons, behavioural responses, vasodilatation and contraction of many smooth muscles.

Chemistry and Distribution

Tachykinins are a family of peptides from ten to twelve residues long. The tachykinin family is characterized by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2, where X is either an aromatic or an aliphatic amino acid. The genes which produce tachykinins encode precursor proteins called preprotachykinins, which are spliced apart into smaller peptides by post-translational proteolytic processing. Substance P and neurokinin A are derived from the same gene TAC1 and occur mainly in nervous system, particularly in the nociceptive sensory neurons, enteric neurons and many regions of brain. Neurokinin B is derived from a separate gene TAC3. The most notable tachykinin is Substance P.

Mechanism of action

Three distinct receptor subtypes of tachykinins namely NK_1, NK_2 and NK_3 have been identified. All receptor subtypes are members of the G protein-coupled superfamily and induce the activation of phospholipase C. The cellular responses occur mainly due to formation of inositol triphosphate leading to release of Ca^{++} within the cells. NK_1, NK_2 and NK_3 preferably bind to substance P, neurokinin A and neurokinin B, respectively. However, the receptor selectivity of these peptides is relatively poor, and it is possible that their actions could be mediated by activation of their less preferred receptors. Most of known effects of tachykinins are mediated by NK_1 and/or NK_2 receptors, with much interspecies variation. Less is known about NK_3 receptors and their role seems to be limited.

Physiological and Pharmacological Effects

Tachykinins elicit a wide range of responses on many types of cells including neurons, smooth muscles, vascular endothelium, exocrine glands, mast cells and cells of immune system. The overall pattern of effects produced by tachykinins is similar, but not identical to that of bradykinin or serotonin.

Smooth muscle: Most types of smooth muscles in body contract in response to tachykinins including those of GI tract and airways.

Blood vessels: Blood vessels in body show a mixture of constrictor and dilator responses (endothelium dependent) to tachykinins together with increased permeability leading to oedema formation.

Nervous system: Many neurons show a slow excitatory response to tachykinins. Substance P plays a key part in the transmission of pain impulses from the peripheral receptors to the central nervous system. It is also thought to be the primary neurotransmitter for the transfer of sensory information from the periphery to the spinal cord and brain.

Emesis: Substance P is present in high concentrations in the vomiting centre of the brain. When activated, substance P results in a vomiting reflux.

Other effects: Tachykinin peptides are involved in inflammation. Tachykinins activate mast cells and cause release of histamine. Various exocrine glands including salivary glands are stimulated.

Patho-physiological Roles

The role of tachykinins in normal and pathophysiological processes is becoming clear through the use of specific antagonists. It is suggested that substance P is involved in the nociceptive pathway and in various conditions with an inflammatory component such as arthritis, asthma and inflammatory bowel disease. It is secreted by enterochromaffin tumours and thus plays a role in the carcinoid syndrome.

PHARMACOLOGY OF TACHYKININS

Tachykinins have no therapeutic application. However, several potent antagonists of NK_1 NK_2 and NK_3 receptors belonging to peptide and non-peptide groups have been discovered. Because the substance P-preferring NK_1 receptor is the predominant tachykinin receptor expressed in the brain, most compounds which have been developed for clinical use are substance P-preferring (NK_1) receptor antagonists.

1. NK_1-Receptor Antagonists

Neurokinin 1 (NK_1) receptor antagonists are a novel class of drugs with significant antiemetic, antidepressant and anxiolytic properties. The main use for which these drugs have been applied so far however is as antiemetics, in both human and veterinary medicine.

Aprepitant

Aprepitant is a recently introduced substance P antagonist. It mediates its effect by blocking the neurokinin 1 (NK_1) receptor. It is used mainly for prevention of acute and delayed chemotherapy-induced nausea and vomiting and for prevention of postoperative nausea and vomiting. Aprepitant may also be considered useful in the treatment of cyclic vomiting syndrome. The pharmacology of aprepitant has been discussed in Chapter 43.

Maropitant

Maropitant is a neurokinin 1 (NK_1) receptor antagonist, developed specifically for the treatment of motion sickness and vomiting in dogs. The pharmacology of maropitant has also been discussed in Chapter 43.

Vestipitant

Vestipitant is a selective antagonist for the NK_1 receptor. It is under development as a potential antiemetic and anxiolytic drug.

2. NK_2-Receptor Antagonists

Saredutant and ibodutant act as NK_2 receptor antagonist. Saredutant has been developed as a novel antidepressant and

anxiolytic drug and is presently undergoing clinical trials. Ibodutant is a candidate drug against irritable bowel syndrome and is also undergoing clinical trials.

3. NK_3-Receptor Antagonists

Osanetant

Osanetant is a neurokinin 3 (NK_3) receptor antagonist developed for the treatment of schizophrenia. Another potential application for osanetant is in the treatment of drug addiction in human beings, as it has been found to block the effects of cocaine in animal models. Currently it is undergoing clinical trials.

Talnetant

Talnetant is another NK_3 receptor antagonist being researched for treatment of irritable bowel syndrome. It is also being evaluated as an antipsychotic drug for the treatment of schizophrenia.

CYTOKINES

Cytokines are a large and diverse family of peptides, proteins or glycoproteins produced throughout the body by cells of diverse embryological origin. The types of cell producing cytokines include glial cells of the nervous system and macrophage, lymphocyte, neutrophil, monocyte, fibroblast and endothelial cells of the immune, haematopoietic and some other systems. Various cytokines include interleukins, interferons, tumour necrosis factors, growth factors, chemokines and colony stimulating factors. Each cytokine has a matching cell-surface receptor with cascades of different intracellular signalling mechanisms. Cytokines mainly function as regulators of inflammatory and immune reactions and some are involved in multiplication and differentiation of cells and in repair processes. The physiology, patho-physiology and pharmacology of cytokines have been discussed in detail in Chapter 56.

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CHAPTER 29

LIPID DERIVED AUTACOIDS

Eicosanoids
- Chemistry and nomenclature
- Biosynthesis, distribution and metabolism
- Mechanism of action
- Physiological and pharmacological effects
- Pathophysiological roles
- Pharmacology of eicosanoids
  - Drugs which stimulate activities of eicosanoids
    - Prostaglandin E_1 and analogues
    - Prostaglandin E_2 and analogues
    - Prostaglandin F and analogues
    - Prostaglandin I and analogues
  - Drugs which inhibit activities of eicosanoids
    - Inhibitors of eicosanoids synthesis
    - Inhibitors of eicosanoid receptors

Platelet activating factor
- Chemistry and synthesis
- Mechanism of action
- Physiological and pharmacological effects
- Pathophysiological roles
- Pharmacology of platelet activating factor
  - Drugs which stimulate activities of PAF
  - Drugs which inhibit activities of PAF

Two distinct families of autacoids are derived from membrane phospholipids - eicosanoids and platelet activating factor. These lipid-derived autacoids contribute to a number of physiological and pathological processes including inflammation, haemostasis, thrombosis, smooth muscle tone, parturition, fever and gastrointestinal secretion. Several classes of drugs, including non-steroidal antiinflammatory drugs (NSAIDs) and glucocorticoids (steroids) owe their principal therapeutic effects to blockade of these lipid derived autacoids formation.

EICOSANOIDS

Eicosanoids are a major group of lipid-derived autacoids consisting of prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), lipoxins (LXs) and hepoxilins. These are derived from unsaturated 20 carbon essential fatty acids, primarily arachidonic acid (5,8,11,14-eicosatetraenoic acid; Greek words: *eicosi* = twenty, *tetraenoic* acid = four double bonds), which is a component of membrane phospholipids. Eicosanoids are implicated in the control of many physiological and pathological processes and are among the most important mediators and modulators of the inflammatory reactions. Several groups of drugs, most notably the non-steroidal anti-inflammatory drugs (NSAIDs), produce their therapeutic effects to blockade of the formation of eicosanoids.

Chemistry and Nomenclature

Eicosanoids are ubiquitous substances which are released from cell membrane phospholipids. They are found in plants and animals. Biosynthetically, eicosanoids are derived by oxygenation from 20-carbon polyunsaturated fatty acids (therefore collectively called eicosanoids) containing a total of three, four or five double bonds which are 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid), 5,8,11,14-eicosatetraenoic acid (arachidonic acid) and 5,8,11,14,17-eicosapentaenoic acid, respectively. In mammalian tissues, 5,8,11,14-eicosatetraenoic acid or arachidonic acid is the principal source of eicosanoids. The biosynthesis of eicosanoids is dependent on the availability of arachidonic acid or other polyunsaturated fatty acids.

Prostaglandins: Prostaglandins are a group of eicosanoids derived enzymatically from arachidonic acid. Prostaglandins are so named because early research workers first identified them in seminal fluid and were believed to be part of the prostatic secretions. Chemically, they are considered as derivatives of prostanoic acid, an unsaturated fatty acid that does not naturally occur in body. Prostanoic acid is a 20-carbon carboxylic acid with a cyclopentane (five member) ring. Depending on the ring structure and the substituent on it, prostaglandins have been designated into different series like A, B, C, D, E, F, G, H and I. Each series has members with numeric subscript 1, 2 and 3 indicating the number of double bonds in the side chain. For example, PGE_1 has one double bond at C 13-14, PGE_2 has two double bonds at C 5-6 and C 13-14, and PGE_3 has three double bonds at C 5-6, C 13-14 and C 17-18 (Figure 29.1). Prostaglandins derived from arachidonic acid carry the subscript 2 (because 2 out of four double bonds of arachidonic acid get saturated during cyclisation) and are the major prostaglandins in mammals. For the PGF series, the subscript α or β refers to the configuration of hydroxyl group at carbon 9 of cyclopentane ring. Prostacyclin or prostaglandin I_2 contains a double ring component rather than the single cyclopentane ring. This distinguishes it from other prostaglandins and is therefore sometimes taken as a family distinct from prostaglandins.

Thromboxanes: Thromboxanes are the second group of eicosanoids derived from arachidonic acid. Unlike prostaglandins, they are considered analogues of unsaturated thrombanoic acid. They are synthesized in thrombocytes (platelets) and contain a

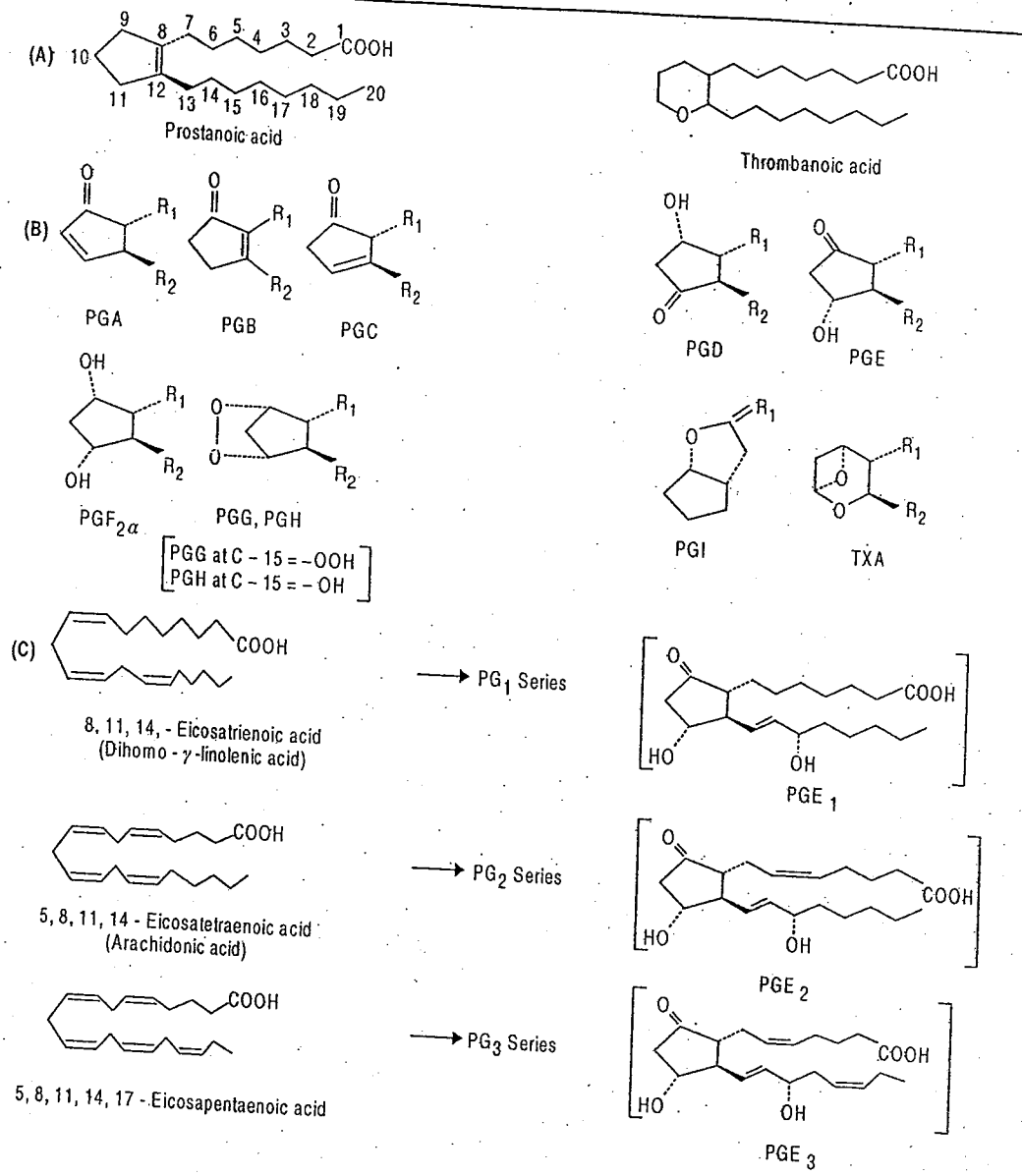


Fig. 29.1. Chemistry and terminology of eicasanoid precursors and eicosanoids.
A : Chemical structures of unnatural fatty acid precursors of prostaglandins and thromboxanes.
B : Basic structure of various prostanoids.
C : Chemical structures of various 20- carbon polyunsaturatted fatty acid precursors of PG_1, PG_2 and PG_3 series prostanoids.

six member oxane ring (therefore called thromboxanes) rather than the cyclopentane ring of prostaglandins. Thromboxanes have been designated like prostaglandins. The most important thromboxane is thromboxane A_2 (TXA_2).

Leukotrienes: Leukotrienes are another important class of eicosanoids derived from arachidonic acid by action of enzymes lipoxygenases. Leukotrienes are so named because they were first found in leucocytes (leuko) and have 3 conjugated double bonds (trienes). Similar to prostaglandins, they have been designated A, B, C, D, E and F and given subscripts 1, 2, 3 and 4. Because no cyclisation or reduction of double bonds occurs during leukotrienes synthesis from arachidonic acid, they carry the subscript 4 (e.g. LTB_4). The LTB_4, LTC_4 and LTD_4 are major leukotrienes in mammals.

Biosynthesis, Distribution and Metabolism

Biosynthesis: Eicosanoids are synthesised from arachidonic acid (5,8,11,14-eicosatetraenoic acid), which is derived from cell membrane phospholipids. In response to various stimuli (physical, chemical, neurological, immunological and hormonal), the enzyme phospholipase A_2 is activated that liberates arachidonic acid from the membrane phospholipids. Once liberated, arachidonic acid is metabolised by several distinct enzyme systems including cyclooxygenases and lipoxygenases, which convert it into a variety of products (eicosanoids) with powerful physiological and pathological activities. Broadly, the pathways of biosynthesis of eicosanoids are divided into two: cyclooxygenase pathway and lipoxygenase pathway.

1. *Cyclooxygenase pathway:* The cyclooxygenase (COX) pathway generates prostaglandins and thromboxanes in a stepwise manner by a complex of microsomal enzymes. The prostaglandins (including prostacyclin) and thromboxanes have a ring structure and are collectively called prostanoids. The first and most important enzyme in this synthetic pathway is prostaglandin endoperoxide synthase, also called prostaglandin G/H synthase, fatty acid cyclooxygenase or simply cyclooxygenase (COX). The cyclooxygenase enzyme exists in three distinct iso-forms COX-1, COX-2 and COX-3. The cyclooxygenase-1 (COX-1) is found in most cells as a constitutive enzyme and is involved in normal homeostasis. The COX-2 is generally not normally present in tissues, but may be induced by certain serum factors, cytokines and growth factors and is considered important in inflammatory and other pathological conditions. Recent evidences have shown that although COX-2 has more important role in inflammatory and other pathological conditions, but it is also constitutively expressed in certain areas of kidney, brain, nerves, ovaries and uterus and appears to be associated with certain physiological functions. COX-3 is the third and most recently discovered cyclooxygenase COX isozyme, and is actively being studied for its similarities and differences between the COX-1 and COX-2 enzymes.

   The prostaglandin G/H synthase or cyclooxygenase enzyme contains two active sites: a cyclooxygenase site and a heme with peroxidase activity site. The cyclooxygenase site of the COX converts arachidonic acid into the cyclic endoperoxide prostaglandin G_2 (PGG_2), which is then converted by its peroxidase moiety to prostaglandin H_2 (PGH_2). The cyclic endoperoxides PGG_2 and PGH_2 are chemically unstable and are immediately transformed enzymatically by isomerases and synthases, present in specific cells, into a variety of terminal prostanoids including prostaglandin I_2 (PGI_2 or prostacyclin), thromboxanes A_2 (TXA_2), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which takes place in different cells. Platelets primarily synthesise TXA_2, whereas vascular endothelium generally generates PGI_2. Some tissues like lung and spleen can synthesise whole range of prostanoids. Prostaglandins A, B, and C are not found in body; they are artefacts formed during extraction procedures.

2. *Lipoxygenase pathway:* Lipoxygenases (LOXs) are a family of non-haem iron containing enzymes which catalyze the oxygenation of polyenic fatty acids to corresponding lipid hydroperoxides. The lipoxygenase pathway system consists of three important sub-pathways catalysed by lipoxygenases 5, 12 and 15. The lipoxygenases 5, 12 and 15 metabolise arachidonic acid to a number of open chain compounds with hydroperoxy group at 5, 12 or 15 positions called 5, 12 and 15 hydroperoxyeicosatetraenoic acid (HPETEs), respectively. All HPETEs are unstable intermediates, which are further metabolised by various enzymes.

   The 5-lipoxygenase (5-LOX) is the most important of lipoxygenase system, which increases synthesis of 5-HPETE and various leukotrienes. Similar to cyclooxygenase enzyme, the 5-lipoxygenase enzyme works in a two-step reaction. In the first step, 5-LOX catalyzes oxidation of arachidonic acid at the 5-position to yield 5-hydroperoxycicosatetraenoic acid (5-HPETE), which in the next step is converted to an unstable 5,6-epoxide known as leukotriene A_4 (LTA_4). The LTA_4 is subsequently transformed into bioactive eicosanoids by multiple pathways. The four most important products formed from leukotrienes A_4 are leukotrienes B_4 (LTB_4), C4 (LTC_4), D_4 (LTD_4) and E_4 (LTE_4). The LTB_4, also called 5,12-dihydroxyeicosatetraenoic acid, is a potent chemotactic agent. The LTC_4, LTD_4 and LTE_4, also called cysteinyl leukotrienes due to presence of cysteine in their structure, constitute products which were originally known as the 'slow-reacting substance of anaphylaxis (SRS-A)'. Leukotrienes are synthesised in neutrophils, monocytes, macrophages, mast cells and keratinocytes, as well as lungs, spleen, brain and heart. The LTB_4 is produced mainly by neutrophils and cysteinyl leukotrienes by eosinophils, mast cells, basophils and macrophages. Phagocytosis and presence of immune complexes in macrophages, mast cell anti-IGE antibodies, and release of platelet activating factor (PAF) by basophils and mast cells are important stimuli for leukotrienes production.

   The 15-lipoxygenase (15-LOX) and 12-lipoxygenase (12-LOX) are other important enzymes which generate unstable 15-HPETE and 12-HPETE. These unstable HPETEs may then be converted to their corresponding hydroxy fatty acids i.e. hydroxyeicosatetraenoic acid (HETE). The 12-HETE leads to formation of epoxy-hydroxyeicosatrienoic acid called hepoxilins (HXs), while 15-HETE forms trihydroxylated metabolites called lipoxines (LXs). At present two lipoxins namely lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) have been identified. In a manner similar to 15-HETE, leukotriene A_4 (LTA_4) can be also be converted by 12 LOX in platelets to lipoxins LXA_4 and LXB_4.

3. *Other pathways:* Arachidonic acid is metabolized also by lesser important pathways. These include an enzymatic pathway called epoxygenase pathway and a non-enzymatic pathway called isoprostane pathway. The epoxygenase pathway involves specific isozymes of microsomal cytochrome P-450 monooxygenases, which metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs). The EETs can be stored in membrane phospholipids (unlike prostanoids) and are important modulators of cardiovascular and renal function. Isoprostanes (isoeicosanoids) are formed non-enzymatically by direct free radical based attack on arachidonic acid and related lipid substrates. Unlike the COX derived eicosanoids, the isoprostanes are formed completely *in situ* on phospholipids and subsequently released pre-formed. It is thought that they may play a role in pathophysiology of inflammatory responses. Steroidal or non-steroidal anti-inflammatory drugs do not block their production.

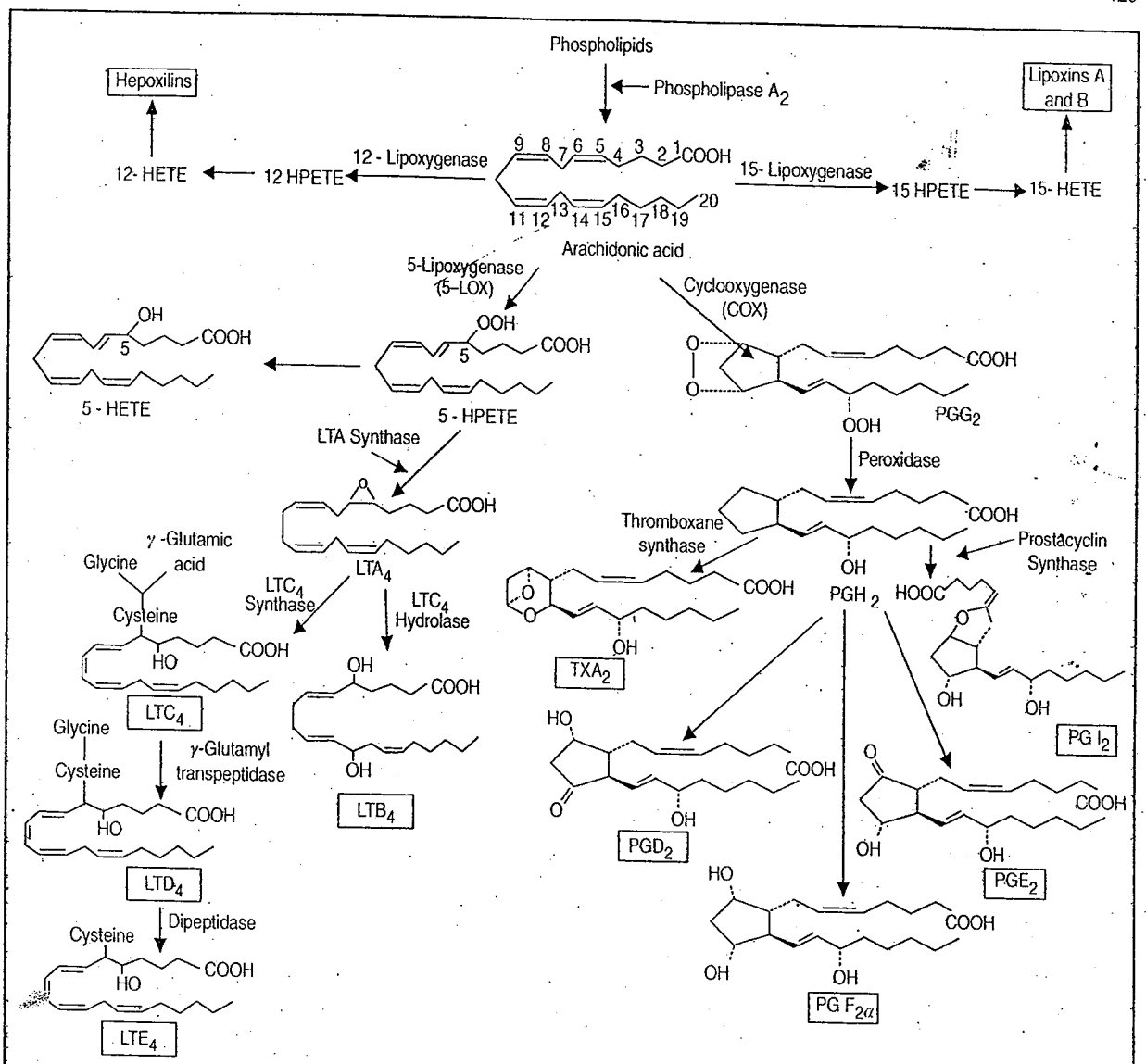


Fig. 29.2. Biosynthesis and chemical structures of eicosanoids (See text for detail). Agents with biological actions are shown in boxes.

Abbreviations : PG = prostaglandin (PGD_2, PGE_2, $PGF_{2\alpha}$, PGG_2, PGH_2, PGI_2) ; TX = thromboxane (TXA_2) ; LT = leukotriene (LTA_4, LTB_4, LDC_4, LTE_4 ; HETE = hydroxyeicosatetraenoic acid ; HPETE = hydroperoxyeicosatetraenoic acid.

------ bonds that lie behind the plane of ring.

▬ bonds that lie in front of the plane of ring.

Distribution and metabolism: Eicosanoids are the most widely distributed autacoids in body. Practically every cell and tissue is capable of synthesizing one or more types of eicosanoids. Unlike many other autacoids, eicosanoids are not localized or stored (except lipoxins and hepoxilins) but are produced by most cells in tissue pools. Instead they are synthesized *de novo* at rates governed by the release of arachidonic acid from membrane phospholipids in response to appropriate stimuli. Most eicosanoids have short half-lives (few seconds to few minutes). They produce short-lived biological activities and are rapidly inactivated. Degrading enzymes are located in most tissue including lungs (degrade about 90% of PGE_2 and $PGF_{2\alpha}$), kidneys, spleen, adipose tissue and intestine. Prostacyclin (PGI_2) has short half-life of about 2-3 minutes and is spontaneously hydrolysed in body fluids to biologically inactive 6-keto-$PG_{1\alpha}$. Thromboxane A_2 has a brief half-life of about 30 seconds under physiological conditions and is converted into a more stable thromboxane B_2. Leukotriene B_4 can be converted to 20-hydroxy-LTB_4, which is further oxidized to 20-carboxy LTB_4. The LTC_4 and LTD_4 are metabolised to LTE_4, which is excreted in urine.

Mechanism of Action

Prostaglandins, thromboxanes, leukotrienes and lipoxins mediate their actions by acting on their own specific receptors located on cell membranes.

Prostanoid receptors: Five major types of prostanoid receptors have been defined, each for the natural prostanoid for which it has the greatest affinity. All prostanoid receptors are G-protein coupled receptors which utilise inositol 1,4,5-triphosphate (IP_3)/diacyl glycerol (DAG) or cAMP transducer mechanisms (Table 29.1). Multiple prostanoid receptors are frequently coexpressed in a single cell type or tissue.

1. ***DP-Receptors:*** Prostaglandin D_2 (PGD_2) acts on DP receptors, although some action of PGE_2 is also noted.

Two subtypes of DP receptors have been identified namely DP_1 and DP_2; DP_1 is the primary receptor mediating most of the prostaglandin D_2 effects. Stimulation of DP_1-receptors by PGD_2 through G_s protein activates adenylate cyclase enzyme causing an increase in intracellular levels of cAMP and Ca^{++} concentration. The DP_1 receptors are expressed in low levels in ileum, lung, stomach, uterus and CNS. The DP_2 receptor coupled with the G_q-PLC-IP_3 pathway causes increase in intracellular Ca^{2+}. It is found on T cells and eosinophils and at the foetal–maternal interface in decidua. The DP_2 expressed on Th2 cells was initially designated as chemo-attractant receptor-TH2 ($CRTH_2$) and the name is still being used on some occasions. The DP receptors are mainly responsible for inhibition of platelet aggregation, relaxation of GI and uterine smooth muscle, and modification of release of hypothalamic/pituitary hormones.

2. ***EP-Receptors:*** EP receptors have highest affinity for prostaglandin E_2 (PGE_2). Based on the physiological and molecular cloning information, EP-receptors have been further subdivided into EP_1, EP_2, EP_3 and EP_4. Prostaglandins EP_1 and EP_2 receptors have limited distribution compared with the distribution of EP_3 and EP_4 receptors.

(*i*) *EP_1 receptors:* The EP_1 receptor is coupled to G_q protein and acts through a phosphatidylinositol-calcium second messenger system. It is mainly responsible for contraction of bronchial and GI smooth muscles. Studies in mice also suggested a role of this receptor in mediating algesia and regulation of blood pressure. In addition to PGE_2, EP_1 receptor is also activated by $PGF_{2\alpha}$.

(*ii*) *EP_2 receptors:* The EP_2 receptor activates the G protein G_s and activates adenylate cyclase to cause an increase in intracellular cAMP. The EP_2 receptor is expressed at much lower levels in most tissues and can be induced in response to inflammatory stimuli. The EP_2 receptors cause smooth muscle relaxation (e.g. bronchodilation and vasodilatation) and stimulation of intestinal fluid secretion.

(*iii*) *EP_3 receptors:* The EP_3 receptors are G-protein coupled receptor existing in 4 isoforms viz. $EP_{3A\text{-}D}$. The EP_{3A}/EP_{3D} isoforms mediate their effects through G_i protein causing inhibition of adenylate cyclase and a decrease in intracellular cAMP. In contrast, EP_{3B}/EP_{3C} receptors coupled with G_s-mediated cause activation of adenylate cyclase enzyme. Some effects of EP_{3D} receptors are also mediated through G_q protein with increase in phospholipase C (PLC) activity. The EP_3 receptor performs several important functions including inhibition of gastric acid secretion, increased gastric mucus secretion, contraction of smooth muscles and inhibition of lipolysis and autonomic neurotransmitter release. It is also involved in digestion, nervous system and kidney reabsorption.

(*iv*) *EP_4 receptor:* The EP_4 receptor is a recently identified receptor coupled to G-protein G_s and mediating its effects through activation of adenylate cyclase enzyme. Knockout studies in mice suggested that EP_4 receptor may be involved in the neonatal adaptation of circulatory system, patency of ductus arteriosus, osteoporosis, as well as initiation of skin immune responses.

3. ***FP-Receptors:*** The FP receptors have greatest affinity for $PGF_{2\alpha}$ and exist in two isoforms viz. FP_A and FP_B. The isoforms of receptor are coupled to G_q protein and mediate IP_3/DAG generation and increase in cytosolic Ca^{++}. The FP receptor is most abundant in the corpus luteum, where its expression pattern varies during the oestrus cycle. It is also expressed in kidneys, hearts, lungs, stomach and eyes. Activation of FP receptors causes myometrial contraction in humans, luteolysis in cattle and bronchoconstriction in cats and dogs.

4. ***IP-Receptors:*** The IP receptors have greatest affinity for prostacyclin (PGI_2), although PGE_1 also appears to act on IP receptors. Signal transduction mechanisms involve activation of adenylate cyclase and an increase in cAMP through G-protein G_s. It is expressed in many tissues and cells, including kidneys, lungs, liver, heart and blood vessels. Activation of IP-receptors causes inhibition of platelet aggregation, renin release, natriuresis and vasodilatation.

5. ***TP Receptors:*** TP receptors are activated by thromboxane A_2 (TXA_2) and prostaglandin endoperoxides (e.g. PGH_2) and exist in two isoforms TP_α and TP_β. The TP receptors are coupled to the G protein G_q. Signal transduction mechanism involves IP_3/DAG generation and increase in cytosolic Ca^{++}. The TP receptors are expressed in platelets, vasculature, lungs, kidneys, heart, thymus and spleen. They have been associated with platelet aggregation and smooth muscle contraction.

Leukotriene Receptors: Three distinct receptors for leukotrienes have been identified in different tissues. These receptors are leukotriene B_4 receptor (BLT), cysteinyl leukotriene receptor 1 (CysLT1) and cysteinyl leukotriene receptor 2 (CysLT2). All leukotriene receptors belong to G-protein superfamily and appear to function through the IP_3/DAG transducer mechanism.

1. ***Leukotriene B_4 receptor (BLT):*** Two distinct types of Leukotriene B_4 receptor viz. leukotriene B_4 receptor 1 (BLT_1) and leukotriene B_4 receptor 2 (BLT_2) have been identified. Leukotriene B_4 binds to BLT_1 and activates the G_i and G_{16} classes of G proteins. The action of BLT_2 appears to be mediated through G_q-like and G_i-like proteins. The BLT_1 expression is restricted to leucocytes; where as the activities of BLT_2 mainly occurs in spleen, followed by liver, ovary and leucocytes. The leukotriene B_4 receptors mediate mainly chemotaxis of granulocytes and macrophages.

2. ***Cysteinyl leukotriene receptor 1 (CysLT1):*** The CysLT1 receptor has greatest affinity for leukotriene D_4 (LTD_4), although leukotrienes C_4 (LTC_4) and E_4 (LTE_4) also appear to act it. The CysLT1 is coupled to G_q protein and the effect is mediated through activation of phospholipase C. Activation of this receptor by LTD_4 results in contraction and proliferation of smooth muscle, oedema, eosinophil migration and damage to the mucus layer in the lung.

Table 29.1: Important features of various types of eicosanoid (prostaglandins, thromboxanes and leukotrienes) receptors.

Feature	DP		EP				$FP_{A,B}$	IP	$TP_{\alpha\beta}$	BLT	CysLT1	CysLT2
	DP_1	DP_2	EP_1	EP_2	EP_{3A-D}	EP_4						
Endogenous agonist	PGD_2	PGD_2	PGE_2, $PGF_{2\alpha}$	PGE_2	PGE_2	PGE_2	$PGF_{2\alpha}$	PGI_2, PGE	TXA_2, PGH_2	LTB_4	LTD_4, LTC_4, LTE_4	LTC_4/LTD_4, LTE_4
Receptor superfamily	G-Protein (G_s)	G-Protein (G_i)	G-Protein (G_q)	G-Protein (G_s)	G-Protein (G_i, G_s, G_q)	G-Protein (G_s)	G-Protein (G_q)	G-Protein (G_s)	G-Protein (G_q)	G-Protein (G_i, G_{16}, G_q)	G-Protein (G_q)	G-Protein (G_q)
Signal transduction	Activation of AC	Inhibition of AC	Activation of PLC	Activation of AC	Activation of AC ; Inhibition of AC ; Activation of PLC	Activation of AC	Activation of PLC	Activation of AC	Activation of PLC	Activation of PLC	Activation of PLC	Activation of PLC
Second messenger	↑cAMP	↓ cAMP; ↑Ca^{++}	↑IP_3/DAG; ↑ Ca^{++}	↑cAMP	↑cAMP; ↓ cAMP; ↑IP_3/DAG	↑cAMP	↑IP_3/DAG; ↑ Ca^{++}	↑cAMP	↑IP_3/DAG; ↑ Ca^{++}	↑IP_3/DAG; ↑ Ca^{++}	↑IP_3/DAG: ↑ Ca^{++}	↑IP_3/DAG; ↑ Ca^{++}
Main effects	Platelet aggregation inhibition, GI and uterine smooth muscle relaxation.	–	Bronchial and GI smooth muscle contraction.	Broncho-dilation, vasodilation, GI smooth muscle relaxation, stimulation of intestinal fluid secretion.	Gastric acid secretion inhibition, gastric mucus secretion activation, smooth muscle contraction, digestion.	Neonatal adaptation of circulatory system, patency of ductus arteriosus, osteoporosis.	Uterine smooth muscle contraction, bronchocons-triction, luteolysis, induction of parturition.	Platelet aggregation inhibition, natriuresis vasodilation.	Platelet aggregation, smooth muscle contraction	chemotaxis of granulocytes and macrophages, hyperalgesia.	contraction and proliferation of smooth muscle, oedema, eosinophil migration.	Role in endocrine and cardiovascular systems.
Agonists	–	–	Iloprost, sulprostone	Butaprost	Sulprostone, misoprostol	Misoprostol	Dinoprost, fluprostenol, latanoprost	Iloprost, cicaprost, carbacyclin	–	–	–	–
Antagonists	–	Ramatroban	–	–	–	–	–	–	Ramatroban	–	Montelukast, zafirlukast	–

Abbreviations: PG = prostaglandin (PGD_2, PGE_2, $PGF_{2\alpha}$, PGH_2, PGI_2), TXA_2 = thromboxanes A_2; LT = leukotriene (LTB_4, LTC_4, LTD_4, LTE_4); cAMP = 3', 5'-cyclic adenosine monophosphate; IP_3 = 1, 4, 5-inositol triphosphate; DAG = diacyl glycerol.
↑ = increase; ↓ = decrease.

3. *Cysteinyl leukotriene receptor 2 (CysLT2):* The leukotriene C_4/D_4 (LTC_4/LTD_4) is the primary ligand for CysLT2 receptor, although leukotriene E_4 (LTE_4) also acts on it as a secondary ligand. The CysLT2 is a member of the superfamily of G protein-coupled (G_q) receptors mediating its action by activation of phospholipase C. It seems to play a major role in endocrine and cardiovascular systems.

Lipoxin receptors: Lipoxins as well as certain peptides are high affinity ligands for the lipoxin A_4 receptor (LXA4R), which was first identified based on sequence homology as the formyl peptide receptor like receptor (FPRL1). Lipoxin signalling through the lipoxin A_4 receptor inhibits chemotaxis, transmigration, superoxide generation and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation. The NF-κB is a protein complex that controls the transcription of DNA.

Physiological and Pharmacological Effects

Prostanoids (PGs and TXs)

Different types of prostanoids have wide variety of actions in body, which may be complimentary or opposite to each other. There is also lot of variations on the species, tissues, hormonal status and other factors. Nevertheless, the general action of prostaglandins and thromboxanes can be expressed in following ways:

Cardiovascular system: The effect of prostaglandins on cardiovascular system is complex. The PGE_2 and PGI_2 cause relaxation of vascular smooth muscle leading to vasodilatation in most, but not all, vascular beds. The PGI_2 is uniformly vasodilatory and appears to maintain blood flow to vital organs. It is about five times more potent than PGE_2 in producing this effect. Responses to $PGF_{2\alpha}$ vary with species and vascular bed; it has a constricting effect on veins and pulmonary vasculature but can cause vasodilatation elsewhere. The TXA_2 consistently produces vasoconstriction. The cyclic endoperoxides (PGG_2 and PGH_2) are potent vasoconstrictors, but they often produce vasodilatation or a biphasic response due to rapid conversion to other prostaglandins, especially to PGI_2 in the vascular endothelium.

The PGE_2 stimulates heart by weak direct but more prominent reflex action due to fall in blood pressure. The cardiac output increases.

Digestive system: The effect of prostaglandins on GI smooth muscles is also complex. The longitudinal muscle is contracted by PGE_2 and $PGF_{2\alpha}$, while the circular muscle is contracted by PGI_2 and $PGF_{2\alpha}$ and relaxed by PGE_2. Prostaglandins reduce transit time in the small intestine and colon. The prostaglandins E_2 and $F_{2\alpha}$ stimulate the movement of water and electrolytes into the intestine, whereas PGI_2 prevents this. Prostaglandin E_2 acts directly on the GI tract and causes inhibition of gastric acid secretion and stimulation of gastric mucus secretion.

Respiratory system: The respiratory smooth muscle is contracted by TXA_2 and $PGF_{2\alpha}$, but relaxed by PGE_1 PGE_2 and PGI_2. The PGE_2 and PGI_2 also inhibit histamine release. Asthmatics are more sensitive to the constrictor as well as dilator effects of prostanoids.

Reproductive system: The PGE_2 and $PGF_{2\alpha}$ contract uterine smooth muscle, especially in gravid uterus near term. Prostaglandin $F_{2\alpha}$ produced in the uterus function as a luteolytic hormone in some subprimate species including cattle. In males, PGE_1 enhances penile erection by relaxing the smooth muscle of the corpora cavernosa. The role of prostaglandins in semen is conjectural.

Kidney: Prostanoids play a major role in maintaining renal physiology, hemodynamics, glomerular filtration, tubular function and urine formation by both renovascular and tubular effects. PGE_2 and PGI_2 increase water, Na^+ and K^+ excretion and have a diuretic effect. They cause renal vasodilatation and inhibit tubular reabsorption. In contrast, TXA_2 causes renal vasoconstriction with a decline in renal function.

Platelets: Eicosanoids play a major role in platelet aggregation. Small concentrations of thromboxane (TXA_2) and prostaglandin E_2 promotes platelet aggregation, while high concentrations of PGE_2, PGD_2 and PGI_2 inhibit platelet aggregation. Damage to endothelial cells usually causes formation of haemostatic plug as a result of a decrease in PGI_2 synthesis in vascular endothelium and an increase in TXA_2 synthesis by platelets (see for detail Chapter 35). TXA_2 also amplifies signals for other platelet agonists such as thrombin and adenosine diphosphate.

Nervous system: Fever is inducted by a number of pyrogens by releasing interleukins which promotes the synthesis and release of PGE_2. The PGE_2 mediates bacterial and other pathogen induced fever at the level of hypothalamus. PGD_2 induces natural sleep by acting on arachinoid trabecular cells in the basal forebrain to increase extracellular adenosine during intracerebral administration. Prostaglandins may also be functioning as neuromodulators in the brain by regulating neuronal excitability.

Endocrine system: The PGE-type prostaglandins inhibit release of norepinephrine from sympathetic neurons and may promote release of growth hormone, prolactin, thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle stimulating hormone (FSH) and luteinising hormone (LH). $PGF_{2\alpha}$ induces an oxytocin dependent decline in progesterone level in parturition.

Pain: Prostaglandins contribute to pain both centrally and peripherally. PGE_2 and PGI_2 lower the pain threshold and sensitises nociceptors to mediators of pain (see for detail Chapter 30).

Eye: Prostaglandin $F_{2\alpha}$ induces constriction of the iris sphincter muscle, but its overall effect in the eye is to decrease intraocular pressure (IOP) by increasing the aqueous humour outflow of the eye via the uveoscleral and trabecular meshwork pathway.

Bone: Prostaglandins are strong modulators of bone metabolism. PGE_2 stimulates bone formation and resorption through osteoblastic and osteoclastic activities affecting bone strength and composition.

Metabolism: Prostaglandins (PGEs) inhibit lipolysis and have some insulin like-effect on carbohydrate metabolism. They also exert parathyroid hormone like-effects resulting in mobilisation of calcium from bones.

Leukotrienes

Cardiovascular system: The leukotrienes markedly increase capillary permeability and cause exudation of plasma. In this aspect, LTC_4 and LTD_4 are reported to be more than 1000 fold more potent than histamine. The LTD_4 and LTC_4 have no

significant effect on most large arteries but they may cause hypotension as a result of decrease in intravascular volume due to increased capillary permeability.

Blood: The LTB_4 is a potent chemotactic agent for polymorphonuclear leucocytes, eosinophils and monocytes. The LTB_4 also promotes neutrophils adhesion to and migration through the vascular endothelium.

Inflammation: Leukotrienes play a major role in inflammation and immune responses. Leukotrienes are generally proinflammatory with LTB_4 a potent chemoattractant for polymorphonuclear leucocytes, eosinophils and monocytes and LTC_4 and LTD_4 chemotactic for eosinophils. LTB_4 promotes aggregation of polymorphonuclear leukocytes, degranulation, generation of superoxide, adhesion and migration. It also stimulates synthesis of cytokines from macrophages and lymphocytes. The leukotrienes stimulate T cell clonal expansion by stimulating the formation of interleukin 1 and 2 as well as the expression of interleukin 2 receptors.

Smooth muscles: The cysteinyl leukotrienes (e.g. LTC_4 and LTD_4) contract most smooth muscles. They induce bronchoconstriction in most species and induce spastic contractions of GI tract at low concentrations. They also stimulate bronchial mucus secretion and cause mucosal oedema.

Nervous system: Like prostaglandins, LTB_4 causes sensitisation of nociceptors to mediators of pain. The release of prostaglandins and LTB_4 during inflammation process thus serves as an amplification system for pain mechanism.

Lipoxins

Lipoxins are primarily anti-inflammatory. They cause activation of monocytes and macrophages, and inhibit activation of neutrophils, eosinophils and lymphocytes. They also inhibit natural killer cell cytotoxicity. Lipoxins serve as high affinity antagonists to the cysteinyl leukotriene receptor type 1 (CysLT1) to which several leukotrienes (LTC_4, LTD_4 and LTE_4) mediate their smooth muscle contraction and eosinophil chemotactic effects.

Hepoxilins

Hepoxilins are pro-inflammatory in skin, but anti-inflammatory in neutrophils. They are also potent insulin secretagogues. Cells under oxidative stress are reported to secrete hepoxilins, which in turn upregulate peroxidase activity thereby decreasing the oxidative stress. This is proposed to constitute a compensatory defence response to protect the vitality and functionality of the cell.

Pathophysiological Roles

The eicosanoids play diverse and important physiological and patho-physiological roles in the body.

1. ***Cardiovascular system:*** Locally generated PGE_2 and PGI_2 modulate vascular tone. These prostaglandins also have been implicated in the maintenance of patency of the ductus arteriotus and in maintaining placental blood flow during foetal life.
2. ***Inflammatory and pain response:*** Prostaglandins and leukotrienes are released by mechanical, thermal, chemical, bacterial and other stimuli and contribute importantly to the genesis of signs and symptoms of inflammation. Prostaglandins (also LTB_4) probably serve as algesic agents during inflammation and they sensitise nociceptors to mediators of pain. The PGE_2 released in hypothalamus mediates pyrogen-induced fever.
3. ***Bronchoconstriction and asthma:*** Many prostaglandins and leukotrienes are released during allergic bronchoconstriction and asthma. It is suggested that in asthma there may be an imbalance between the bronchoconstrictor eicosanoids ($PGF_{2\alpha}$, PGD_2, TXA_2, LTC_4 and LTD_4) on one hand and bronchodilator mediators (PGE_2 and PGI_2) on the other.
4. ***Platelets and thrombosis:*** Prostaglandin endoperoxides (PGG_2 and PGH_2) and TXA_2 play important role in platelet aggregation and development of thrombosis, while PGI_2 prevents these events.
5. ***Reproduction and parturition:*** In females, $PGF_{2\alpha}$ causes luteolysis in subprimate species (e.g. cattle) and causes contraction of uterine smooth muscles. It has been postulated that prostaglandins mediate initiation and induction of parturition and labour. In males, prostaglandins have been associated with erection, ejaculation, sperm motility and steroidogenesis.
6. ***Kidneys:*** Prostaglandins modulate renal blood flow and may serve to regulate urine formation. They also regulate secretion of renin.
7. ***Gastrointestinal tract:*** Prostaglandins E_2 and I_2 reduce acid secretion and appear to be involved in the secretion of mucus in stomach (PGE_2) and regulation of gastric mucosal blood flow (PGI_2). Therefore, they may be functioning as natural ulcer protective agents.
8. ***Endocrine system:*** Prostaglandins, especially PGE_2, facilitate release of several hypothalamic/anterior pituitary hormones and insulin.
9. ***Cancer:*** Some eicosanoids have been associated with development of cancer and malignancies. Angiogenesis is promoted by TXA_2, PGE_2 and PGI_2 and specific COX-2 expression is associated with many tumours such as colon cancer and breast cancer.

PHARMACOLOGY OF EICOSANOIDS

Two distinct classes of drugs are important in modulating eicosanoid signalling: those which stimulate activities of eicosanoids by acting as receptor agonists and those which inhibit activities of eicosanoids either by blocking their synthesis or acting directly as receptor antagonists.

DRUGS WHICH STIMULATE ACTIVITIES OF EICOSANOIDS

I. PROSTAGLANDINS AND ANALOGUES

Several synthetic prostaglandins or their analogues are available commercially for use in veterinary and human medicines. These synthetic compounds are more stable than the natural agents and some are effective orally.

1. Prostaglandin E_1 and Analogues

Alprostadil

Alprostadil is a synthetic preparation of prostaglandin E_1. It is used mainly in human medicine to increase pulmonary blood flow and oxygenation of blood in infants with congenital heart defects till surgery is undertaken. The pulmonary blood vessels, particularly the ductus arteriosus in neonates are sensitive to vasodilatory effect of prostaglandin E_1. Alprostadil administered either as a suppository or injected intracavernously into the

penis causes erection in impotent patients who do not have disorders of vascular system or cavernous body damage.

Misoprostol

Misoprostol (15-deoxy-16-hydroxy-16 methyl PGE_1) is a synthetic analogue of prostaglandin E_1. It occurs as a yellow viscous liquid having a musty odour. It is used both in veterinary and human medicines as an ulcer-healing agent.

Pharmacological effects

Misoprostol has a cytoprotective and antiulcer effect on the gastric mucosa. It inhibits basal, nocturnal and food-induced gastric acid secretion by a direct action on gastric parietal cells. It also increases production of gastric mucus and bicarbonate and enhances blood supply to gastric mucosal cells. Misoprostol appears to heal gastric ulcers about as effectively as the H_2 receptor antagonists; however, it does not provide relief from ulcerogenic pain (see also Chapter 42).

Misoprostol increases amplitude and frequency of uterine contractions and stimulate uterine bleeding.

Pharmacokinetics

Misoprostol is rapidly and effectively absorbed from the GI tract. It is rapidly metabolised to misoprostol acid, which is the primary active metabolite. Misoprostol acid is further biotransformed to pharmacologically inactive metabolites. Small amounts of misoprostol, free acid and inactive metabolites are excreted mainly in the urine.

Side effects/Adverse effects

Important side effects seen with misoprostol include diarrhoea, nausea, vomiting, abdominal cramps and flatulence. Potentially uterine contractions and vaginal bleeding may occur in bitches. Adverse effects are often transient and may resolve with continued therapy or by dosage adjustment. Administration of drug along food may decrease chances of side effects.

Contraindications and precautions

Like other prostaglandins, misoprostol is contraindicated during pregnancy due to its uterine stimulant activity. Abortions and birth defects are important adverse effects of misoprostol when it is administered during pregnancy. Misoprostol should be used cautiously in patients sensitive to prostaglandins and in cerebral or coronary vascular disease. Pregnant women workers should avoid exposure to misoprostol.

Drug interactions

Non-systemic antacids reduce absorption of misoprostol and may reduce its bioavailability. Magnesium containing antacids may aggravate misoprostol-induced diarrhoea.

Clinical uses

In veterinary medicine, misoprostol may be useful as primary or adjunctive therapy in treating or preventing gastric ulceration, especially when induced or aggravated by non-steroidal anti-inflammatory drug. It is less effective in duodenal ulcers and reflux oesophagitis. Misoprostol is also considered effective for treating pruritus in patients with atopic dermatitis, although it is less effective than other drugs. Misoprostol is available in 0.1 mg and 0.2 mg tablets.

Dose

Dogs : 2 – 5 µg/kg, PO, 2-3 times daily.
Horses : 0.5 µg/kg, PO, 4 times daily.

Other PGE_1 drugs

Several other synthetic analogues of prostaglandin E_1 are available commercially. These include enisoprost, gemeprost, limaprost and rioprostil. Enisoprost and rioprostil are newly developed synthetic analogues of prostaglandin E_1 possessing significant gastric antisecratory activity, considered effective in the therapy of gastric ulcers and to provide protection against NSAID-induced gastric mucosal damage. Gemeprost (16, 16-dimethyl-trans-delta2 PGE_1 methyl ester) is used as a treatment for obstetric bleeding. Limaprost (17α,20-dimethyl-Δ2-Prostaglandin E_1) is an analogue of PGE_1 with structural modifications intended to give it a prolonged half-life and greater potency. All these drugs are used in human medicine and have no/negligible usage in veterinary practice.

2. Prostaglandin E_2 and Analogues

Dinoprostone

Dinoprostone is a synthetic prostaglandin E_2. It acts by binding and activating the prostaglandin E_2 (EP) receptor. Dinoprostone promotes ripening and dilation of cervix, and causes contraction of uterus. It also stimulates osteoblasts to release factors which stimulate bone resorption by osteoclasts. Dinoprostone is primarily used as an abortifacient in human medicine. It is used primarily for induction/augmentation of labour or mid-term abortion. Dinoprostone comes as a vaginal insert and a gel for insertion into the vagina. Side/Adverse effects are not common, but may include GI disorders (Vomiting and diarrhoea), dizziness and fever.

Sulprostone

Sulprostone is a metabolism resistant synthetic analogue of PGE_2. It is a selective agonist for EP_3 receptors. Sulprostone is a potent stimulator of uterine smooth muscle contractions with high abortifacient activity. It is mainly used in human medicine for induction of labour or mid-term. It may also be used in some cases of post-partum haemorrhage due to uterine atonia.

Other PGE_2 drugs

Albaprostil, enprostil, metenprost and trimoprostil are other synthetic analogues of prostaglandin E_2. These are primarily used in human medicine and have no/negligible usage in veterinary practice. Butaprost, a selective EP_2 agonist has frequently been used to pharmacologically define the EP receptor expression profile of various human and animal tissues and cells.

3. Prostaglandin F and Analogues

Dinoprost

Dinoprost is prostaglandin $F_{2\alpha}$, which is commercially available as tromethamine (THAM) salt. Dinoprost tromethamine, also known as dinoprost trometamol, prostaglandin $F_{2\alpha}$ or $PGF_{2\alpha}$ occurs as a white to off-white, very hygroscopic crystalline powder. It is widely used in veterinary medicine for luteolytic and ecbolic actions.

Pharmacological effects

Dinoprost tromethamine has primary action on the female reproductive system. It causes stimulation of uterus, relaxation of cervix and regression of corpus luteum (luteolysis). Induction of luteolysis depends on the dose, frequency of drug administration and stage of cycle. In mares, cows and ewes, the corpus luteum is refractory to the action of $PGF_{2\alpha}$ or analogues for up to 5 days post-ovulation; in sows the refractor-

up to 11 days and in bitches and queens the corpus luteum in generally unresponsive at any time after ovulation unless subject to repeated doses.

Pharmacokinetics

The pharmacokinetic data on dinoprost are limited in domestic animals. The drug is reported to distribute very rapidly to tissues after injection. Its half-life in serum is reported to be only few minutes in cattle. It follows probably the same metabolic pathway as discussed earlier for endogenous prostaglandins.

Side effects/Adverse effects

Prostaglandin $F_{2\alpha}$ or analogues are very potent compounds with a wide range of side/adverse effects in man and animals. Important side/adverse effects of dinoprost in animals include transient sweating, decreased body temperature, tachycardia, tachypnoea, abdominal pain and ataxia in horses; transient increased body temperature, tachypnoea, hypersalivation, restlessness, diarrhoea and urination in pigs; and transient salivation, increased body temperature, restlessness, tremor and mild diarrhoea in cattle. Abdominal pain, nausea, vomiting, diarrhoea, hypersalivation, tachypnoea and panting in dogs, and vocalisation, mydriasis and possible emesis and diarrhoea in cats are significant adverse effects. The adverse effects of $PGF_{2\alpha}$ in small animals are mainly dose related but signs abate after repeated dosing. Occasionally, dinoprost may produce severe reactions at the site of IM injection, sometimes leading to death.

Overdosage of dinoprost or $PGF_{2\alpha}$ analogues may produce severe adverse effects which are multiplication of side effects outlined above.

Treatment: Symptoms of overdosage of $PGF_{2\alpha}$ should be treated by supportive therapy.

Contraindications and precautions

Dinoprost should not be used during pregnancy in all species, unless termination of pregnancy is required. Dinoprost is contraindicated in animals with bronchoconstrictive respiratory disease (e.g. asthma) and disorders of vascular system. Dinoprost or $PGF_{2\alpha}$ analogues should be carefully handled by human operators because they can be absorbed through skin and may produce bronchospasm or miscarriage. Therefore, pregnant women, asthmatics and persons with bronchial problems should avoid contact with or wear protective gloves when administering the drug. Accidental spillage should be washed off immediately with soap and water.

Drug interactions

Dinoprost should not be administered along with other oxytocic agents because of enhancement of pharmacological effects. Concomitant use of progestin may reduce efficacy of dinoprost and other related agents.

Clinical uses

Dinoprost is widely used in veterinary practice as a luteolytic agent to induce predictable onset of oestrus or synchronisation of oestrus in a variety of species. It may also be used for termination of pregnancy or for induction of parturition either alone or in combination with corticosteroids (cattle and sheep) or dopaminergic agents (dogs). In pigs, dinoprost has been used to induce parturition when given within 3 days of farrowing. In addition to above conditions, $PGF_{2\alpha}$ or analogues have been found useful in treatment of a number of pathological conditions including pyometra, mummified foetus and luteal cysts. The drug is preferably administered after fasting in the dogs and cats to decrease incidence of vomiting.

Dose

For oestrus synchronisation

Cattle : 25 mg (total), IM, once or twice at a 10-12 day interval.

Sheep & goats : 8 mg (total), IM, on day 4-5 of oestrous cycle and repeated in 11 days.

For termination of pregnancy (as abortifacient)

Cattle : 25 – 30 mg (total), IM, between 5-150 days of gestation.

Swine : 5 – 10 mg (total), IM.

Sheep : 10 – 15 mg (total), IM, during first two months of pregnancy.

For induction of parturition

Cattle : 25 – 30 mg (total), IM. Parturition usually occurs 72 hours of injection.

Swine : 10 – 25 mg (total), IM, 2 to 6 days before expected parturition. Farrowing usually occur within 24 to 36 hours of injection.

Goats : 2.5 – 5 mg (total), IM on day 144. Parturition usually occurs within 1 to 3 days.

For pyometra

Dogs & cats : 0.25 mg/kg, SC, once daily for 5 days.

Cattle : 25 mg (total), IM. Uterus begins evacuating within 24 hours.

Cloprostenol

Cloprostenol sodium is a synthetic analogue of $PGF_{2\alpha}$. Similar to dinoprost, cloprostenol is a powerful luteolytic agents. It causes rapid regression of the corpus luteum and arrests its secretory activity. It also has a direct stimulating effect on uterine smooth muscle causing its contraction with accompanying a relaxant effect on the cervix. In this regard, cloprostenol is more potent than $PGF_{2\alpha}$. Cloprostenol is mainly approved for use in beef and dairy cattle to induce luteolysis. It is also used for chronic endometritis, expulsion of mummified foetus, luteal cysts, abortions after mismating, and synchronisation of oestrus. It is not recommended for dogs and cats; however, studies regarding use of drug in small animals are under investigation. Its side/adverse effects, contraindications and precautions are similar to those of dinoprost.

Dose

For induction of oestrus

Cattle : 0.5 mg (total), IM. Oestrus usually commences in 2 to 5 days.

Horses : 0.25 – 0.5 mg (total), IM.

For induction of parturition

Cattle : 0.5 mg (total), IM.

Swine : 0.175 mg (total), IM.

Goats : 0.0625 – 0.125 mg (total), IM at 144 days of gestation.

For termination of pregnancy (as abortifacient)

Cattle : 0.5 mg (total), IM, before 150 days of pregnancy.

Horses : 0.25 – 0.5 mg (total), IM, before 35 days of pregnancy.

For pyometra/chronic endometritis

Cattle : 0.5 mg (total), IM.

Etiproston

Etiproston tromethamine is a synthetic analogue of prostaglandin $F_{2\alpha}$. It is a luteolytic agent indicated for parenteral use in cows and sows. Following parenteral administration, etiproston is rapidly absorbed from the injection sites. In cattle, peak plasma level of the drug in reached in 30 min to 2 hour following intramuscular injection. Absorption from GI is limited and hence the route is not used for etiproston administration. Etiproston is extensively metabolised in liver and is largely excreted in urine. The side/adverse effect and therapeutic profile of etiproston is in general similar to dinoprost.

Dose

Cattle : 5 mg (total), IM.

Other $PGF_{2\alpha}$ drugs

Several other synthetic $PGF_{2\alpha}$ analogues are available for use in veterinary and/or human practice. These include veterinary drugs alfaprostol, carboprost, fenprostalene, fluprostenol, luprostiol, tiaprost and travoprost. Like dinoprost and cloprostenol, alfaprostol, fenprostalene, fluprostenol, luprostiol and tiaprost are primarily used for induction of oestrus, induction of parturition, metritis and pyometra in domestic animals. Carboprost tromethamine is primarily used in human medicine for inducing uterine contractions and triggering abortion in early pregnancy (13–20 weeks of gestation); it is also recommended in postpartum haemorrhage caused by uterine atony not controlled by other methods. Travoprost is used topically as an ophthalmic solution for controlling the progression of glaucoma or ocular hypertension; it reduces intraocular pressure by increasing the outflow of aqueous fluid from the eyes.

Dose

Fluprostenol

Horses : 0.25 mg (total), IM.

Luprostiol

Cattle : 15 mg (total), IM.

Horses : 7.5 mg (total), IM.

Tiaprost

Swine : 0.3 – 0.6 mg (total), IM. (for parturition)

4. Prostaglandin I and Analogues

Epoprostenol

Epoprostenol is the pharmaceutical preparation of prostacyclin (PGI_2). Epoprostenol inhibits platelet activation and prevents formation of the platelet plug involved in primary haemostasis. It is also an effective vasodilator. It has short half-life and is used by IV infusion to inhibit platelet aggregation during haemodialysis, especially if heparin if contraindicated. It is also used for improving the harvest and storage of blood platelets for therapeutic transfusion.

Ioprost

Iloprost is a synthetic analogue of prostacyclin PGI_2. It is primarily used to treat pulmonary arterial hypertension (PAH), scleroderma and ischaemia. Iloprost dilates systemic and pulmonary arterial vascular beds. It also affects platelet aggregation but the relevance of this effect to the treatment of pulmonary hypertension is unknown. Hypotension, cough, headache, nausea, neck spasms and insomnia are some common side/adverse effects observed in human patients. Iloprost is mainly used in human medicine by intravenous, subcutaneous or inhalation route.

Other PGI_2 drugs

Some other synthetic analogues of prostacyclin (PGI_2) include beraprost, treprostinil, cicaprost and carbacyclin. The major effects of beraprost and treprostinil are vasodilatation of arteries in the pulmonary (lung) and body and inhibition of platelet aggregation. Treprostinil is clinically indicated for the treatment of pulmonary arterial hypertension, whereas beraprost is presently undergoing clinical trials for the treatment of pulmonary hypertension. Cicaprost is metabolically stable and bioavailable after oral administration. Carbacyclin is a chemically stable analogue of prostacyclin causing inhibition of platelet aggregation and reduction of systemic arterial blood pressure (BP). However, it is much less active than prostacyclin.

DRUGS WHICH INHIBIT ACTIVITIES OF EICOSANOIDS

1. Inhibitors of Eicosanoids Synthesis

Glucocorticoids

Glucocorticoids are an important class of therapeutic agents which inhibit the release of arachidonic acid from membrane phospholipids, which in turn inhibits synthesis of all eicosanoids (e.g. prostaglandins, thromboxanes and leukotrienes). This effect is mediated indirectly by stimulation of synthesis of a peptide called lipocortin, which blocks phospholipase A_2, the enzyme responsible for synthesis of arachidonic acid. Glucocorticoids are widely used in therapeutic for anti-inflammatory and other varieties of effects and are discussed separately in Chapter 51.

Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are an important class of therapeutic agents which inhibit cyclooxygenase enzyme (COX) and block synthesis of prostanoids (prostaglandins and thromboxanes). Inhibition of COX, in fact, increases production of leukotrienes by promoting availability of substrate (arachidonic acid) to the lipoxygenase pathways. Non-steroidal anti-inflammatory drugs are widely used in both human and veterinary medicines to treat inflammation, mild to moderate pain and fever. The pharmacology of NSAIDs has been discussed separately in Chapter 30.

Zileuton

Zileuton is an inhibitor of 5-lipoxygenase, the enzyme that generates leukotrienes (LTB_4, LTC_4, LTD_4 and LTE_4) from arachidonic acid. Zileuton was introduced in 1996 and is now marketed for the prophylaxis and chronic treatment of asthma in human beings. Following oral administration zileuton is rapidly absorbed with a mean time to peak serum concentration of 1.7 hours. Zileuton is 93% bound to plasma proteins, primarily to albumin, with minor binding to alpha-1-acid glycoprotein. It is primarily metabolised by the cytochrome P-450 enzymes in liver and is largely excreted as metabolites in the urine. In man, it has an average elimination half-life of 2.5 hours.

Zileuton is well tolerated in normal doses. Occasional side effects reported in human beings with oral formulations are nausea, sinusitis and pharyngolaryngeal pain. The drug is a minor inhibitor of some cytochrome P-450 isoenzymes and has been shown to increase the serum concentration or effects of theophylline, propranolol and warfarin. Zileuton is contraindicated in patients with active liver disease as it is reported to increase liver enzymes. Zileuton is available for oral administration in immediate release and extended release formulations. The use of zileuton in veterinary practice is not reported.

Piriprost

Piriprost is a structural analogue of prostaglandin I_2 (PGI_2) with low IP receptor-mediated activity. Piriprost primarily inhibits 5-lipoxygenase enzyme and blocks the synthesis of leukotrienes. Piriprost is also reported to inhibit some release of histamine. Similar to zileuton, the piriprost has shown some efficacy in the treatment of asthma and other inflammatory conditions in human beings. The literature on the therapeutic uses of piriprost in veterinary is not reported.

Dazoxiben

Dazoxiben (4-(2-(1H-Imidazol-1-yl)ethoxy)-benzoic acid) is a selective thromboxane synthase enzyme inhibitor. Inhibition of thromboxane synthase by dazoxiben decreases the plasma concentration of thromboxane A (TXA_2) and hence platelet aggregation. Thromboxane A_2 is also a potent vasoconstrictor and hence its decreased plasma concentration causes vasodilatation. These actions of dazoxiben are considered useful in the prevention of pulmonary hypertension. Dazoxiben has also shown useful effects in septic shock conditions, where the levels of thromboxane B_2 (TXB_2), the stable metabolite of thromboxine A_2, have been found to increase. In some experimental conditions, the dazoxiben has also been used during haemodialysis and found considerably useful. The overall therapeutic usefulness of dazoxiben, however, is not clear.

Pirmagrel

Pirmagrel is another selective thromboxane synthase enzyme inhibitor. Its pharmacological action is similar to dazoxiben. Similar to dazoxiben, its clinical usefulness is not clear.

Ridogrel

Ridogrel is a combined thromboxane synthase inhibitor and thromboxane receptor antagonist. Ridogrel is a more potent antiplatelet agent than aspirin because it inhibits thromboxane synthesis directly and also blocks the thromboxane receptor, while the aspirin inhibits the cyclooxygenase only, the enzyme responsible for producing thromboxane. Ridogrel is clinically used with streptokinase as an adjunctive therapy to reduce the formation and size of blood clots in conditions such as acute myocardial infarction. Overall, the clinical experience with ridogrel is still relatively limited.

ETYA

ETYA (5,8,11,14-eicosatetraynoic acid) is a non-metabolizable structural analogue of arachidonic acid (5,8,11,14-eicosatetraenoic acid). Due to structural similarity to arachidonic acid, ETYA is acted upon by cyclooxygenases and lipoxygenases, but instead of metabolizing it both enzymes themselves get blocked. ETYA is reported to modulate voltage gated potassium current and also Ca^{2+} entry into cells. ETYA also stimulates luteinizing hormone release from cultured pituitary cells. ETYA is an experimental drug used for research purposes only.

2. Inhibitors of Eicosanoids Receptors

A few eicosanoids receptor antagonists have been developed and are available for clinical use. These agents include montelukast and zafirlukast, both $CysLT_1$ receptor antagonists). The selective antagonists of TP receptors (e.g. sulotroban and vapiprost) and EP receptors are not available for clinical use.

Montelukast

Montelukast is a recently introduced leukotriene CysLT1 receptor antagonist. By inhibiting CysLT1 receptor, montelukast

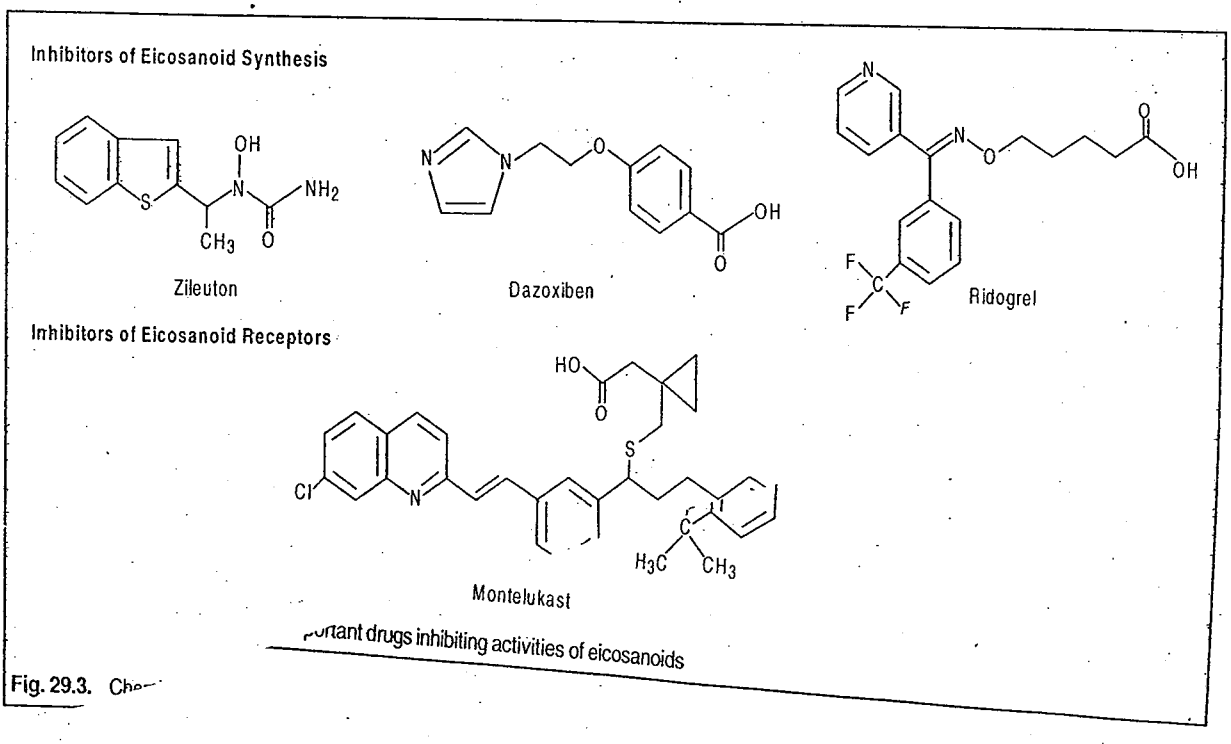


Fig. 29.3. Chemical structures of important drugs inhibiting activities of eicosanoids

blocks the action of leukotriene D_4 (primary ligand) and also LTC_4 and LTE_4 (secondary ligands). The effect is significant in the respiratory tract including bronchioles and lungs. This reduces the inflammatory conditions in respiratory tract and decreases bronchoconstriction otherwise caused by the leukotrienes.

Montelukast has been indicated for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. However, it is not considered useful for the treatment of acute asthma attacks. Important side/adverse effects associated with montelukast in human beings include gastrointestinal disturbances, hypersensitivity reactions, sleep disorders and increased bleeding tendency. Montelukast is usually administered orally, but its detailed pharmacokinetic and therapeutic profiles are not available. There is no literature on the clinical use of montelukast in veterinary medicine.

Zafirlukast

Zafirlukast (Accolate) is a leukotriene CysLT1 receptor antagonist with pharmacological actions and therapeutic uses similar to those of montelukast. It is often used in conjunction with an inhaled steroid and/or long-acting bronchodilator for the maintenance treatment of asthma. The pharmacology of zafirlukast has been discussed in Chapter 46.

Ramatroban

Ramatroban is a thromboxane A_2 (TXA_2) receptor antagonist. It is a potent TP receptor antagonist with excellent efficacy against allergic rhinitis. Recent studies have revealed that ramatroban can also block the newly identified PGD_2 receptor, the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2). PGD_2 induces migration and degranulation of eosinophils through CRTh2 and contributes to late-phase inflammation and cell damage. Accordingly, it was considered that ramatroban suppresses the late-phase inflammation via TP receptor and CRTh2 blockade. Some studies have also suggested that ramatroban can suppress the expression of monocyte chemoattractant protein-1 (MCP-1) and adhesion molecules in endothelial cells and prevent exacerbation of inflammation by blocking these responses. Ramatroban is indicated for the treatment of coronary artery disease and has also been used for the treatment of asthma.

PLATELET ACTIVATING FACTOR

Platelet activating factor (PAF) or acetyl-glyceryl-ether-phosphoryl choline (AGEPC) is a cell membrane derived autacoid. Platelet activating factor is a potent phospholipid activator and mediator of many leukocyte functions, including platelet aggregation (therefore popularly called platelet activating factor), inflammation and anaphylaxis.

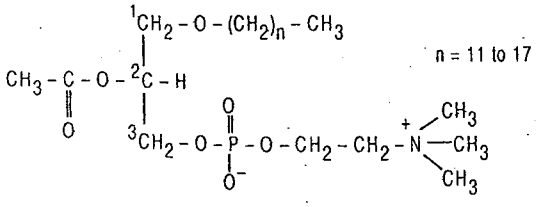


Fig. 29.4. Structure of platelet-activating factor.

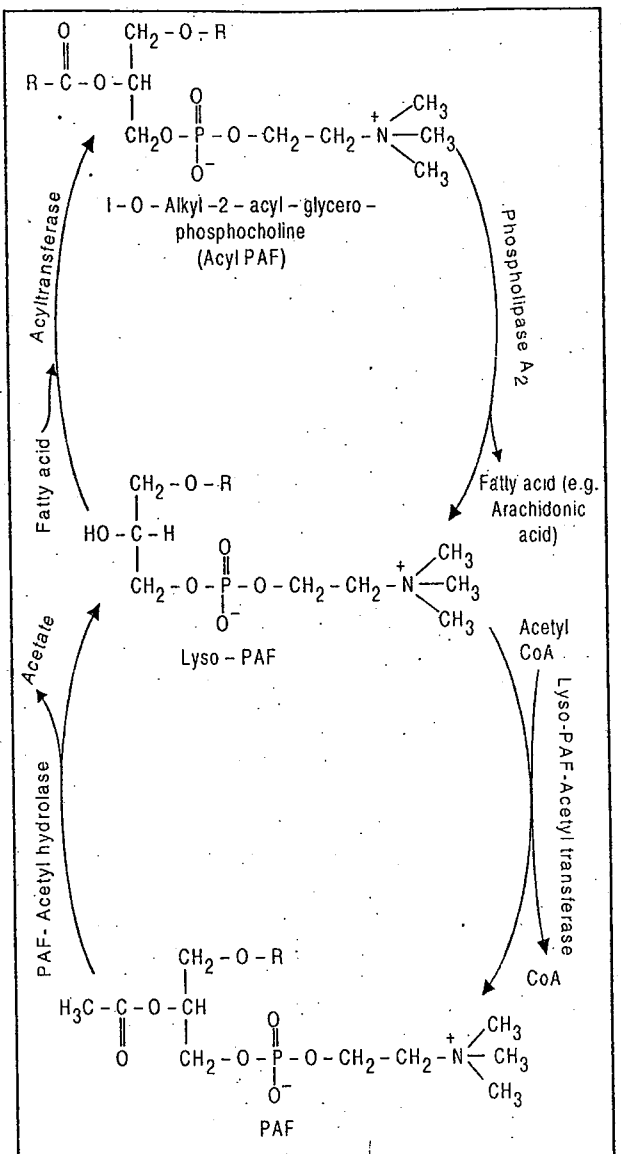


Fig. 29.5. Biosynthesis and degradation of platelet-activating factor. (see text for detail).
Abbreviations: PAF = platelet activating factor; COA = coenzyme A; R = fatty acid.

Chemistry and Synthesis

Synthesis: Chemically PAF is 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine. The PAF actually represents a family of phospholipids because the alkyl group at position 1 can vary in length from 12 to 18 carbon atoms (Figure 29.3). Like eicosanoids, PAF is synthesised from phospholipids in cell membrane by phospholipase A_2. The precursor phospholipid, 1-O-alkyl-2-acyl-glycerophosphocholine, is present in high concentrations in the membranes of many types of cells. Activation of phospholipase A_2 (PLA_2) results in formation of 1-O-alkyl-2-lyso-glycerophosphocholine (lyso-PAF), which in turn is acetylated by enzyme lyso-PAF acetyltransferase to form PAF (Figure 29.4). The second step is rate limiting. In the formation of lyso-PAF, a free fatty acid (usually arachidonic acid) is released, which may serve as source of eicosanoids.

In addition to the enzymatic routes mentioned above, PAF-like molecules can be formed by oxidative fragmentation of membrane phospholipids (OxPLs) in presence of oxidative stress.

They are structurally different from PAF, but bind to the same receptor and elicit the same responses. Unlike the synthesis of PAF, which is highly controlled, OxPLs production is unregulated.

Metabolism: The metabolism of PAF occurs in two steps. In the first step, the acetyl-group of PAF is removed by PAF-acetylhydrolase to form lyso-PAF, which is then converted to the 1-O-alkyl-2-acyl-glycerophosphocholine. Like eicosanoids, PAF is not stored in cells but is synthesised in response to stimulation. Antigen-antibody reaction or a variety of agents including chemotactic peptides, thrombin, collagen and autacoids stimulate PAF synthesis in a Ca^{++} dependent manner. In contrast to eicosanoids, the types of cell which synthesise PAF are quite limited, mainly platelets, neutrophils, monocytes, eosinophils, mast cells, vascular endothelium and kidney cells.

Mechanism of Action

In most cases, PAF appears to exert its action through membrane bound specific G-protein coupled receptors. Activation of PAF receptors coupled to G-protein G_q causes activation of phospholipases C, D and A_2 with resultant formation of inositol phosphates, diacyl glycerol and arachidonic acid. The arachidonic acid released by PAF is converted to eicosanoids (prostaglandins, thromboxanes and leukotrienes), which may function as extracellular mediators of the effect of PAF. The platelet aggregatory action of PAF is believed to be due to unmasking of fibrinogen binding sites on the surface of platelets. PAF also acts intracellularly within the cell where it is synthesized. Accumulation of PAF within cell is associated with the adhesion of neutrophils to the surface of endothelial cells.

Physiological and Pharmacological Effects

Cardiovascular system: Platelet activating factor on IV injection lowers peripheral vascular resistance, causes vasodilatation and produces fall in blood pressure. Decreased coronary blood flow has been observed on intracoronary injection, probably due to platelet aggregation. Similar to histamine and bradykinin, PAF increases vascular permeability and promotes movement of fluid out of vasculature. The increase in vascular permeability is due to constriction of venular endothelial cells. The PAF is approximately 1000-fold more potent than histamine or bradykinin in increasing vascular permeability and is the most potent agent known in this aspect.

Blood: Platelet activating factor causes aggregation of platelets, polymorphonuclear leucocytes and monocytes. The platelet aggregatory action of PAF is considered independent of TXA_2 or other aggregatory factors, although it causes release of TXA_2. Intravascular injection of PAF results in intravascular thrombosis and thrombocytopenia. PAF is chemotactic for eosinophils, neutrophils and monocytes. PAF stimulates neutrophils to aggregate, to stick to vascular endothelium, and to migrate across to the site of infection. It also stimulates polymorphonuclear leucocytes to release leukotrienes and lysosomal enzymes and to generate superoxide radical. It induces degeneration of eosinophils. PAF causes leukocytopenia.

Smooth muscles: Platelet activating factor causes contraction of respiratory, gastrointestinal and uterine smooth muscles. In addition, it produces mucosal oedema, secretion, erosions and and long-lasting bronchial-hyper-reaction soon after IV mucosal bleeding have [illegible] action of myometrium directly injection of PAF

or may promote release of PGE_2 and promote uterine contraction indirectly.

Kidneys: PAF dilates renal afferent arterioles at low concentrations due to stimulation of nitric oxide production by the endothelium. At higher concentrations PAF constricts the vessels by production of COX products.

Pathophysiological Roles

Platelet activating factor is a mediator in a number of physiological and pathological events in the body. Derangement and dysregulation of PAF functioning has been associated with some diseases.

1. *Inflammation:* Platelet activating factor has proinflammatory actions. It is elaborated by leucocytes and mast cells at the site of inflammation and appears to participate in causation of vasodilatation, vascular permeability and exudation, cellular infiltration and hyperalgesia. PAF contributes to the pathophysiology of several inflammatory disorders, including skin diseases.
2. *Allergic responses and anaphylaxis:* PAF is reported to be involved in some allergic and anaphylactic reactions. Along with leukotrienes (LTC_4 and LTD_4), PAF appears to play a major role in bronchial asthma. It causes bronchoconstriction, mucosal oedema and secretions. High circulating PAF levels are associated with anaphylactic and shock conditions.
3. *Thrombosis:* PAF stimulates platelet aggregation and promotes haemostasis and thrombosis. In this aspect, PAF does not function independently but contributes in manner analogues to thromboxanes (TXA_2) and adenosine diphosphate (ADP).
4. *Reproduction and parturition:* PAF may be involved in ovulation, fertilization, implantation and parturition. Early embryos which produce PAF have greater chance of implanting, and PAF produced by foetus at term may help initiation or progression of parturition. PAF may be involved in progression of labour by directly contracting the uterus and releasing PGE_2. The PAF antagonists delay labour in animals.

PHARMACOLOGY OF PLATELET ACTIVATING FACTOR

Drugs Stimulating Activities of PAF

Unlike some eicosanoids, stimulation of PAF activity serves no therapeutic purpose. Platelet activating factor or analogues has no pharmacological or therapeutic roles.

Drugs Inhibitting Activities of PAF

Inhibitors of PAF-Biosynthesis

The anti-inflammatory actions of glucocorticoids are due, at least in part, to inhibition of PAF biosynthesis by virtue of their inhibitory action on phospholipase A_2 (see also Chapter 51).

Inhibitors of PAF-Receptors

[illegible] are reported to inhibit binding of PAF to its Sors and block its action selectively. These include ginkgolide B, a terpene isolated from Chinese tree *Ginkgo biloba*, some triazolobenzodiazepines (e.g. alprazolam and triazolam) and rupatadine, an anti-histamine. The clinical utility of these PAF antagonists is not much clear.

Rupatadine

Rupatadine is a recently introduced second generation antihistamine and PAF receptor antagonist. Rupatadine blocks histamine H_1 and PAF receptors and inhibits degranulation of mast cells induced by immunological and non-immunological stimuli. It also produces inhibition of the release of cytokines, particularly of the tumour necrosis factor (TNF) in mast cells and monocytes. Rupatadine possesses anti-allergic properties and has been recommended for the treatment of allergic rhinitis and chronic urticaria in human beings. Some common effects associated with rupatadine in humans include somnolence, headaches and fatigue. There are no reports of rupatadine use in veterinary practice.

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CHAPTER 30 ANTI-INFLAMMATORY, ANTI-PYRETIC AND NON-OPIOID ANALGESIC DRUGS

Steroids/Glucocorticoids
Non-steroidal anti-inflammatory drugs
- Classification
- Important characteristics of NSAIDS
- Mechanism of action
  - Analgesia
  - Antipyresis
  - Anti-inflammatory effect
- Non-selective COX inhibitors
  - Agents with analgesic and marked anti-inflammatory activity
    - Salicylic acid derivatives/Salicylates
    - Pyrazolone derivatives
    - Indole and indene acetic acid derivatives
    - Oxicam derivatives
    - Amino-nicotinic acid derivatives
    - Hetero-aryl acetic acid derivatives
  - Agents with analgesic and mild to moderate anti-inflammatory activity
    - Propionic acid derivatives
    - Anthranilic acid derivatives/Fenamates
    - Sulphonanilide derivatives
    - Alkalones
  - Agents with analgesic and poor anti-inflammatory activity
    - Para-aminophenol derivatives
    - Pyrazolone derivatives
    - Benzoxazocine derivatives
- Selective COX-2 inhibitors
  - Furanone derivatives
  - Pyrazole derivatives
  - Isoxazole derivatives
  - Hetero-aryl acetic acid derivatives
  - Miscellaneous drugs
- Mixed COX and 5-LOX inhibitors

Other anti-inflammatory and chondroprotective drugs
- Dimethyl sulhoxide
- Hyaluronate sodium/Hyaluronic acid
- Glycosaminoglycans
- Orgotein/Superoxide dismutase
- Gold compounds
- Miscellaneous anti-inflammatory agents

Inflammatory process is a part of normal defence mechanism that aids in the repair of damaged tissues. However, widespread or chronic inflammation and pain may become detrimental and interrupt normal functioning. In such cases, use of pharmacological agents is often required to avoid inadequate tissue perfusion, tissue fibrosis, pain, fever and loss of functioning. A large number of drugs acting by different mechanisms are used clinically in both medical and veterinary practices to decrease intensity of inflammation and to alleviate pain. Many of these agents also lower elevated body temperature and result in a reduction of fever. These drugs have been broadly divided into steroidal (glucocorticoids) and non-steroidal anti-inflammatory drugs. Some other groups of drugs like antihistamines also suppress intensity of inflammation and oedema formation and relieve pain, but they are often discussed elsewhere.

STEROIDS/GLUCOCORTICOIDS

Glucocorticoids, commonly called steroids or steroidal drugs, are an important class of anti-inflammatory drugs which suppress all aspects of acute and chronic inflammatory processes and are extensively used for treatment of various inflammatory conditions. The exact mechanisms by which glucocorticoids act are unknown but they reduce release or synthesis of several inflammatory mediators, including most and possibly all cytokines, complement components, platelet activating factor, eicosanoids (prostaglandins, leukotrienes and thromboxane), and generation of toxic free radicals from neutrophils and macrophages. One important biochemical pathway blocked by corticosteroids is the arachidonic acid cascade (both cyclooxygenase and lipoxygenase pathways) by inhibiting the activity of phospholipase A_2 enzyme (Figure 30.1), but this action does not account for all anti-inflammatory actions of steroids. Disorders of the musculoskeletal system for which glucocorticoids have been used include traumatic arthritis and myositis, bursitis, tendinitis, osteoarthritis and rheumatoid arthritis. These drugs are also widely used for various allergic and non-allergic inflammatory conditions of respiratory system, skin, ear and eye. Other indications include ulcerative colitis, autoimmune haemolytic anaemia and cerebral oedema. Injudicious and excessive use of corticosteroids, however, has risks of serious side effects. The detailed pharmacology and clinical uses of glucocorticoids have been described separately in Chapter 51.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs, usually abbreviated to as NSAIDs, are a heterogeneous group of drugs having weak

or mild analgesic effect with or without antipyretic and anti-inflammatory actions. They act primarily on peripheral pain mechanisms and also in CNS to raise pain threshold. Unlike opioid analgesics, these drugs have no affinity for opioid receptors and do not induce central depressant and sedative activity. Non-steroidal anti-inflammatory drugs do not produce physical dependence and no tolerance develops to these drugs. Since all drugs with the exception of a few (e.g. paracetamol) exhibit anti-inflammatory activity and are not steroids, they are popularly known as non-steroidal anti-inflammatory drugs or NSAIDs. Presently, NSAIDs are among the most widely used of all therapeutic drugs.

Classification

The non-steroidal anti-inflammatory drugs can be classified on the basis of the inhibition of cyclooxygenase isozymes, the analgesic, antipyretic and anti-inflammatory activities, and the chemical structures.

A. Non-selective COX Inhibitors

I. Agents with analgesic and marked anti-inflammatory activity

1. Salicylic acid derivatives/Salicylates
   e.g. aspirin, sodium salicylate, diflunisal, salicylic acid, magnesium salicylate, salsalate and benorilate.
2. Pyrazolone derivatives
   e.g. phenylbutazone, suxibuzone, oxyphenbutazone, azapropazone, phenazone, amidopyrine and ramifenazone.
3. Indole and indene acetic acid derivatives
   e.g. etodolac, indometacin and sulindac.
4. Oxicam derivatives
   e.g. piroxicam, meloxicam, tenoxicam, droxicam and lornoxicam.
5. Amino-nicotinic acid derivatives
   e.g. flunixin and clonixin.
6. Hetero-aryl acetic acid derivatives
   e.g. diclofenac, ketorolac, eltenac, aceclofenac and tolmetin.

II. Agents with analgesic and mild to moderate anti-inflammatory activity

1. Propionic acid derivatives
   e.g. naproxen, carprofen, ketoprofen, vedaprofen, ibuprofen, fenoprofen, indoprofen, flurbiprofen and oxaprozin.
2. Anthranilic acid derivatives/Fenamates
   e.g. meclofenamic acid, tolfenamic acid, mefenamic acid, flufenamic acid, niflumic acid and etofenamic acid.
3. Sulphonalinide derivatives
   e.g. nimesulide.
4. Alkalones
   e.g. nabumetone.

III. Agents with analgesic and poor anti-inflammatory activity

1. Para-aminophenol derivatives
   e.g. paracetamol, phenacetin and acetanilide.
2. Pyrazolone derivatives
   e.g. metamizole and propyphenazone.
3. Benzoxazocine derivatives
   e.g. nefopam.

B. Selective COX-2 Inhibitors

1. Furanone derivatives
   e.g. rofecoxib and firocoxib.
2. Pyrazole derivatives
   e.g. celecoxib, deracoxib and mavacoxib.
3. Isoxazole derivatives
   e.g. valdecoxib and parecoxib.
4. Hetero-aryl acetic acid derivatives
   e.g. lumiracoxib and robenacoxib.
5. Miscellaneous
   e.g. etoricoxib.

C. Mixed COX and 5-LOX inhibitors
   e.g. tepoxalin and licofelone.

The non-steroidal anti-inflammatory drugs are chemically diverse, but almost all are organic acids. The classical (older) NSAIDs were previously divided on the basis of chemical structures into two main groups: carboxylic acids (R-COOH) and enolic acids (R-COH). The carboxylic acids include drugs belonging to salicylates, indolines, amino-nicotinic acids, propionic acids, anthranilic acids and phenylacetic acids (e.g. paracetamol). The enolic acids belong to groups of pyrazolones and oxicams. The selective COX-2 inhibitors, also called COXIBs, may be having differing structures to classical NSAIDs.

Important Characteristics of NSAIDs

NSAIDs as a group tend to have similar characteristics with little difference in mechanism of action and clinical efficacy at equivalent doses. The important differences among compounds tend to be with regards to dosing regimens (related to elimination half-lives), route of administration and side effects. Some important properties of non-steroidal anti-inflammatory drugs are as follow:

a. They are primarily weak organic acids.
b. They possess anti-inflammatory, antipyretic and analgesic effects, although there are important differences in their activities.
c. Most NSAIDs mediate their primary action through inhibition of cyclooxygenase enzyme leading to reduced synthesis of prostaglandins and related compounds.
d. They provide only symptomatic relief from the inflammation and pain associated with disease and do not arrest the progression of pathological injury to tissue during severe cases.
e. They are mostly effective against pain of low to moderate intensity and are more useful in inflammatory pain and those associated with musculo-skeletal system.
f. Their pharmacokinetics varies between species, which leads to marked inter-species differences in dosage requirements.
g. Most NSAIDs are well absorbed after oral administration with bioavailability ranging from 50 to 100%.
h. With the exception of few (e.g. salicylates), they are highly bound to plasma proteins (> 95%), which limits their extra vascular penetration and distribution to tissues and body fluids. High protein binding may not

limit but enhance their therapeutic efficacy in acute inflammation because of protein leaks from vascular compartment into inflamed tissues.

i. Most NSAIDs penetrate blood-brain barrier to act on CNS.

j. Elimination of NSAIDs primarily occurs due to metabolism in liver, mainly by conversion to inactive metabolite, although some (e.g. phenylbutazone and aspirin) also form active metabolites.

k. Many NSAIDs show prolonged duration of action despite their short plasma half-life. This may occur due to irreversible inhibition of cyclooxygenase enzyme (e.g. aspirin), accumulation of drug in inflammatory exudates from where they are cleared more slowly than from plasma, and/or adsorption of drugs onto hay/food from which they are slowly and continuously released and absorbed (e.g. flunixin).

l. Their adverse effects are mostly related to the gastrointestinal tract (e.g. GI irritation and possibly ulceration). All NSAIDs exert action on GI tract to varying degree.

m. Combination of two NSAIDs confers no advantage over high doses of a single drug; rather combination usually results in enhanced toxicity.

n. They show important drug interactions, especially with drugs which are highly bound to plasma proteins.

o. Elimination of NSAIDs primarily occurs due to metabolism in liver, mainly by formation of inactive metabolite, although some (e.g. phenylbutazone and aspirin) form active metabolites.

p. Their toxicity data cannot be transposed between species.

q. Unlike opioid analgesics, they do not show respiratory depression, drug dependence, tolerance or withdrawal symptoms.

Mechanism of Action

The non-steroidal anti-inflammatory drugs act primarily by inhibiting the synthesis of prostaglandins, the mediators involved in nociception and pathogenesis of inflammation and fever. These drugs block cyclooxygenase (COX) enzyme either reversibly (e.g. most NSAIDs) or irreversibly (e.g. aspirin) causing inhibition of synthesis of prostaglandins (PGs), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) (Figure 30.1). This mechanism of action was elucidated by John Vane (1927–2004), who later received a Nobel Prize for his work in 1982. As discussed earlier in Chapter 29, cyclooxygenase enzyme occurs in two isozymes: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The COX-1 is a constitutive enzyme and is considered to be necessary for normal homeostasis, while COX-2 is mainly inducible (induced by cytokines such as IL-1 in inflammation) and is considered to be associated with inflammation and pain. Most NSAIDs inhibit both COX-1 and COX-2 non-selectively, although ratio for the inhibition of COX-1 : COX-2 varies widely being high for aspirin, phenylbutazone and piroxicam, and low for naproxen, carprofen and meloxicam (Table 30.1). Some newer NSAIDs (e.g. celecoxib, rofecoxib and firocoxib) selectively inhibit COX-2. The relatively bulky structure of COX-2 inhibitors prevents COX-1 inhibition

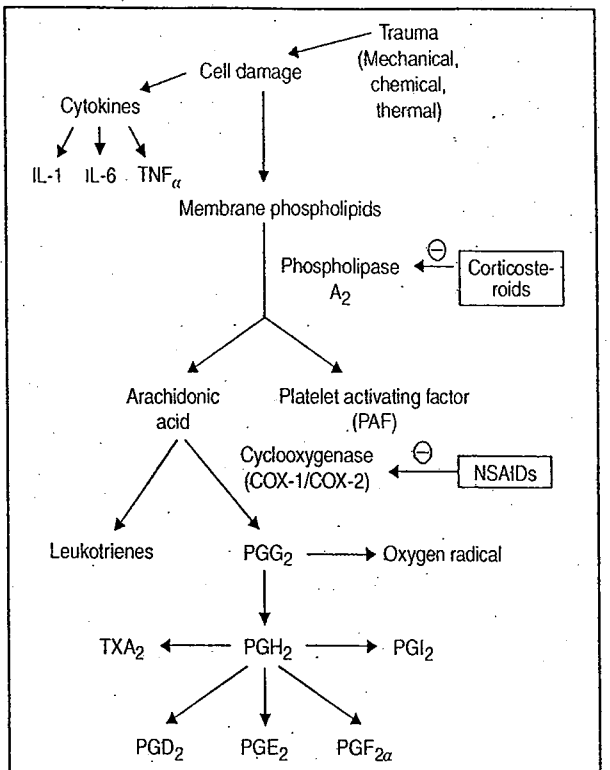


Fig. 30.1. Schematic representation of pathways for production of prostaglandins and some other pro-inflammatory mediators following cell damage, and site of action of non-steroidal antinflammatory drugs.

Abbreviations : NSAIDs = non-steroidal antiinflammatory drugs; PG = prostaglandin ; TX = thromboxane ; COX = cyclooxygenase; IL = interleukin ; TNF = tumour necrosis factor.

by steric hindrance. Most of the toxic effects (e.g. GI irritation, renotoxicity and interference with clotting mechanism) of non-selective inhibitors of COX-1 and COX-2 are due to inhibition of COX-1, while desirable anti-inflammatory and analgesic actions are attributable to blockade of COX-2. However, recently it has been shown that COX-2 also has some constitutive functions associated with renal, nerve, brain, ovaries, uterus and bone; therefore the associated side effects with these systems may not be completely eliminated with selective COX-2 inhibitors.

Other proposed mechanisms of action for some (but not all) NSAIDs include inhibition of neutrophils function, blockade of oxygen radical production, stabilization of lysosomal enzymes, antagonism of bradykinin release, modulation of nitric oxide synthesis, alteration of signal transduction mechanisms, or inhibition of metalloproteinase activity. Some NSAIDs (e.g. aspirin) are reported to influence cytokines production. Non-steroidal anti-inflammatory drugs (except licofelone), however, do not inhibit lipoxygenase production and hence do not suppress leukotrienes formation; rather there may be more formation of leukotrienes due to more availability of arachidonic acid substrate.

Analgesia

Non-steroidal anti-inflammatory drugs produce analgesic effect mainly by inhibiting production of prostaglandins and by decreasing sensitisation of pain receptors to noxious stimuli. Prostaglandins (mainly PGE_2 and PGI_2) sensitize the nociceptive afferent nerve terminals to mediators like bradykinin, cytokines (e.g. TNF-α, IL-1 and IL-8) and some other algesic agents, so that even a small concentration of algesic agent produces intense

Table 30.1: Effect of some non-steroidal anti-inflammatory drugs on COX-1 and COX-2.

NSAID	COX-1	COX-2	COX-2 IC_{50}/COX-1 IC_{50}*
Aspirin	++++	+	166
Phenylbutazone	++++	+	-
Etodolac	+	+++	0.2
Indometacin	+++	+	60
Piroxicam	+++	++	10
Meloxicam	+	+++	0.1
Flunixin	+++	+	125
Diclofenac	++	+++	0.5
Carprofen	+	+++	0.01
Ketoprofen	+++	+	50
Ibuprofen	+++	+	15
Naproxen	++	++	1
Meclofenamic acid	+	+++	0.07
Nimesulide	+	++++	0.03
Paracetamol	0	0	No effect on either enzyme

Note: The values have been taken from many studies using different assay systems which give a wide range of values for the activities listed above. The values are therefore a rough guide only.

IC_{50} = Inhibitory concentration-50; the concentration that reduces activity of the enzyme to 50% of the initial value.

* = The higher the figure for the ratio, the less the effect on COX-2 and the more the effect on COX-1 (i.e. higher the figure for the ratio, more the concentration required to produce inhibitory effect on COX-2).

pain (Figure 30.2). The capacity of prostaglandins to sensitize pain receptors to mechanical and chemical stimuli appears to result from a lowering of the threshold of the polymodal nociceptors of C-fibres. Non-steroidal anti-inflammatory drugs are, therefore, mainly effective against those types of pain in which prostaglandins act to sensitize nociceptors, namely pain associated with inflammation or tissue damage. NSAIDs do not affect the hyperalgesia or the pain caused by direct action of prostaglandins. Some agents (e.g. aspirin) may also depress pain stimuli at subcortical site (thalamus and hypothalamus).

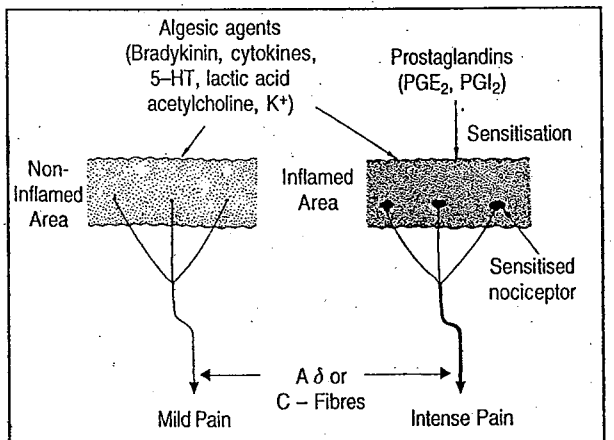


Fig. 30.2. Schematic representation of sensitisation of nociceptors (pain receptors) to algesic agents by prostaglandins at inflammatory sites.

Antipyresis

Normal body temperature is regulated by a centre in the hypothalamus, which ensures a balance between the heat loss and the heat production. During infection (also tissue damage, inflammation or other disease states), there is enhanced generation of cytokines like interleukins (e.g. IL-1β and IL-6), interferons and tumour necrosis factor (TNF-α) which induce PGs production, especially PGE_2, in and near hypothalamic area. The PGE_2 via increase in cyclic AMP triggers the hypothalamus to elevate the set point at which body temperature is regulated resulting in fever. Cytokines either directly or indirectly through induction of PGs (mostly) stimulate also the vasomotor centre in the CNS resulting in sympathetic nerve stimulation, peripheral vasoconstriction, decrease in heat dissipation or fever (Figure 30.3). Non-steroidal anti-inflammatory drugs apparently reset the 'set point or thermostat' and also increase heat loss through peripheral vasodilatation by inhibiting synthesis of prostaglandins. Non-steroidal anti-inflammatory drugs do not influence body temperature when it is elevated by factors such

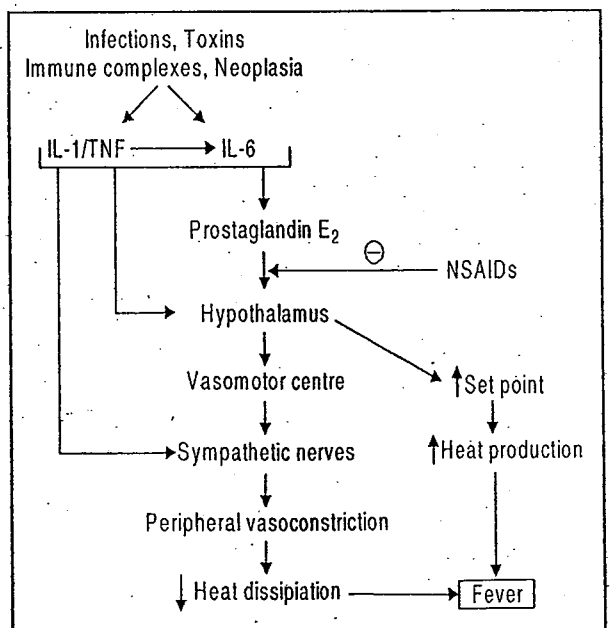


Fig. 30.3. Mechanism of fever.

Abbreviations: IL-1 = interleukin 1, IL-6 = interleukin 6, TNF = tumour neurosis factor.

Table 30.2: Mediators of inflammation.

Mediator(s)	Source	Important functions
Prostaglandins (PGE_2, PGD_2, PGI_2)	Mast cells, from cell membrane phospholipids.	Vasodilatation, sensitisation of nociceptors, facilitation of vascular permeability and fever.
Leukotrienes (LTB_4, LTC_4, LTD_4, LTE_4)	Leucocytes, mast cells and cell membrane phospholipids.	Chemotaxis, leucocyte adhesion, activation of leucocytes and increased production of cytokines.
Platelet Activating Factor (PAF)	Leucocytes, mast cells and membrane phospholipids.	Vascular leakage, vasodilatation, chemotaxis, hyperalgesia, leucocyte priming and bronchoconstriction.
Bradykinin	Plasma substrate.	Vasodilatation, vascular leakage, smooth muscle contraction and pain.
Histamine and Serotonin	Mast cells, platelets.	Vasodilatation, vascular leakage and stimulation of nociceptors (pain production).
Cytokines (Interleukins, TNF-α, β-chemokines)	Macrophages, lymphocytes, fibroblasts, chondrocytes, synoviocytes, eosinophils.	Chemotaxis, acute phase reactions (pyrexia, pain, necrosis), endothelial activation and cartilage degeneration.
Oxygen free radicals (hydroperoxides, superoxide, hydroxyl)	Neutrophils, chondrocytes, macrophages.	Tissue damage, endothelial damage and destruction of invading microorganisms.
Complement Components (C3a, C3b, C5a)	Plasma proteins via liver; macrophages.	Opsonic fragment (C3b) leucocytes adhesion, activation of vascular leakage, chemotaxis and histamine release by mast cells.
Factor XII (Hageman Factor)	Liver	Activates kinin production, fibrinolysis and coagulation system.
Plasmin	Fibrinolysis system	Fibrinolysis, cleavage of complement protein C3 and activation of Factor XII.
Neuropeptides (e.g. substance P, CGRP)	Nerve terminal.	Vasodilatation, vascular leakage, activation of neutrophils and eosinophils, histamine release.
Nitric oxide	Macrophages, chondrocytes, fibroblasts, endothelium, some neurons.	Vasodilatation, cytotoxicity. platelet antiaggregation and leukocyte recruitment.
Neutral proteases	Neutrophils.	Tissue damage.

Abbreviations: PG = prostaglandin; LT = leukotriene; TNF-α = tumour necrosis factor-alpha; CGRP = calcitonin-gene related peptide.

as exercise or rise in ambient temperature. The NSAIDs reduce body temperature in fever, but do not cause hypothermia in normothermic individuals.

Anti-inflammatory effect

Inflammation is a complex process that occurs due to participation of a large number of vasoactive, chemotactic and proliferative factors at different stages. Prostaglandins, leukotrienes, platelet activating factor, cytokines and other mediators (See Table 30.2) are released by a host of mechanical, thermal, chemical, bacterial and other insults which contribute to the genesis of inflammation. Although PGs do not appear to have direct effects on vascular permeability and inflammation, both PGE_2 and PGI_2 markedly enhance oedema formation and leucocyte infiltration by promoting blood flow in the inflamed area. NSAIDs inhibit mainly PGs synthesis at site of injury and produce anti-inflammatory action. However, they do not depress the production of other mediators of inflammation. Certain NSAIDs may act by additional mechanisms including inhibition of expression/activity of some adhesion molecules, stabilisation of leucocyte lysosomal membrane and antagonism of kinins. In general, NSAIDs suppress rather than abolish inflammatory reactions, thereby providing symptomatic relief.

NON-SELECTIVE COX-INHIBITORS

I. AGENTS WITH ANALGESIC AND MARKED ANTI-INFLAMMATORY ACTIVITY

1. Salicylic Acid Derivatives/Salicylates

Aspirin

Aspirin (Acetylsalicylic acid, ASA) is the salicylate ester of salicylic acid and is one of the most extensively used over-the-counter drugs. Bayer first developed aspirin in 1899, and it remains the standard reference for non-opioid analgesics. It occurs as a white crystalline substance. It is stable in dry air but hydrolyses to salicylic acid and acetic acid in moist air. It is slightly soluble

in water and freely soluble in alcohol. Aspirin is often used as an analgesic to relieve minor pains, as an antipyretic to reduce fever, and as an anti-inflammatory drug.

Mechanism of action

Aspirin generally acts by virtue of its content of salicylic acid, although some of its unique effects are due to its capacity to acetylate proteins. As with most other NSAIDs, both the therapeutic and unwanted effects of aspirin and other salicylates are primarily due to inhibition of cyclooxygenase enzyme activity and consequent reduction of PGs-synthesis. Aspirin is unique among the NSAIDs that it irreversibly blocks both COX-1 and COX-2 iso-enzymes. In the structures of COX-1 and COX-2 isoenzymes, aspirin acetylates serine at position 530 and 516, respectively, preventing the binding of arachidonic acid to the active site of the enzyme and thus the ability of enzyme to form prostaglandins. The duration of effects of aspirin are related to the turnover rate of cyclooxygenases in different target tissues. Low-dose, long-term aspirin use irreversibly blocks the formation of thromboxane A_2 in platelets, producing an inhibitory effect on platelet aggregation. This anticoagulant property makes aspirin useful for reducing the incidence of heart attacks.

Aspirin has been shown to have some additional modes of action. It uncouples oxidative phosphorylation in cartilaginous (and hepatic) mitochondria. Therefore when used in high doses, aspirin may actually cause fever due to the heat released from the electron transport chain, as opposed to the antipyretic action of aspirin seen with low doses. Aspirin also induces the formation of NO-radicals in the body, which have an independent mechanism of reducing inflammation. More recent data suggest that salicylic acid and its derivatives modulate signalling through NF-κB, a transcription factor complex that plays a central role in many biological processes, including inflammation. At high doses, aspirin inhibits the activation of inflammatory cells, including neutrophils.

Pharmacological effects

Aspirin acts both centrally and peripherally to produce its pharmacological effects.

Analgesic effects: Aspirin possesses weak to moderate analgesic effect as compared to morphine-like drugs. It relieves inflammatory, rheumatic, tissue injury-related, connective tissue, muscular, arthritic and integumental pain, but is relatively ineffective is severe visceral and ischemic pain. The analgesic effect is mainly peripheral in the form of blockade of pain impulse generation and prevention of PGs mediated sensitisation of nerve endings. Central analgesia has been ascribed to inhibition of actions of PGs in causing facilitation and perpetuation of the actions of spinal transmitters. A central subcortical action raising threshold to pain perception also contributes, but morphine like action or psychic processing or reaction component of pain is absent. Aspirin and other NSAIDs have no effect on the reticular activating system or the limbic system.

Antipyretic effect: Aspirin and other salicylates have marked antipyretic action and they reduce the elevated body temperature. As discussed earlier, aspirin resets the hypothalamic thermostat and rapidly reduces fever by increasing heat loss as a result of peripheral vasodilatation, panting and sweating. Aspirin does not decrease heat production. It has no effect on normal body temperature.

Anti-inflammatory effect: Aspirin exerts anti-inflammatory action at relatively high doses. It inhibits signs of inflammation such as pain, tenderness, swelling, vasodilatation and leucocytes infiltration. It also reduces vascular permeability and oedema formation. Aspirin inhibits inflammation in arthritis, but it neither arrests the progress of disease nor does it induce the remission.

Respiration and acid-base balance: At therapeutic doses, aspirin stimulates alveolar ventilation. At high doses, it increases both respiratory rate and volume by direct stimulation of respiratory centre and by uncoupling oxidative phosphorylation resulting in respiratory alkalosis. The respiratory alkalosis is usually compensated by kidneys through excretion of bicarbonate accompanied by an increased excretion of sodium and potassium. Toxic doses or prolonged administration can depress the medulla, resulting in respiratory acidosis followed by metabolic acidosis due to accumulation of organic acids.

Gastrointestinal tract: As discussed earlier, the prostacyclin (PGI_2) inhibits gastric acid secretion and PGE_2 and $PGF_{2\alpha}$ stimulate synthesis of protective mucus in both the stomach and small intestine. In the presence of aspirin, these prostaglandins are not formed, resulting in increased gastric acid secretion and diminished mucus protection. This may cause epigastric distress, nausea and vomiting. The ion trapping of drug in gastric mucosal cell (aspirin remains unionised and diffusible in gastric acid but on entering the mucosal cell it ionises and becomes non-diffusible) enhances gastric irritation and toxicity. Aspirin produces a dose-related gastric ulceration and haemorrhage. The gastric bleeding may be painless and frequently leads to blood loss in the stool and occasionally to ion-deficiency anaemia.

Haematological effects: Aspirin, even in small doses, irreversibly inhibits the platelet cyclooxygenase enzyme and the subsequent synthesis of thromboxane A_2, a potent platelet aggregator. As a result of decreased TXA_2 synthesis, platelet aggregation time is reduced producing an anticoagulant effect with a prolonged bleeding time. Because platelets lack nucleus, they cannot synthesize new enzyme and the lack of thromboxane persists for the lifetime of platelets (3 to 7 days). Therefore, aspirin has more persistent antithrombotic actions than other NSAIDs. Low doses of aspirin are more effective in reducing platelet aggregation than high doses because in low doses aspirin inhibits synthesis of TXA_2 only, while in high doses it also blocks synthesis of prostacyclin, a platelet anti-aggregator, which reduces the overall platelet antiaggregatory effect of the drug. Long-term use of large dose decreases synthesis of clotting factors in liver and predisposes patient to bleeding.

Renal effects: Aspirin and other NSAIDs prevent synthesis of PGE_2 and PGI_2 in the kidneys which are responsible for maintaining renal blood flow, particularly in the presence of circulating vasoconstrictors. Decreased synthesis of PGs can result in retention of sodium and water, and may cause oedema and hyperkalaemia.

Cardiovascular effects: Therapeutic doses of aspirin have no direct effect on cardiovascular system. The peripheral blood vessels dilate after large doses due to direct effect on vascular smooth muscle. Toxic doses depress vasomotor centre causing fall in blood pressure.

Metabolic effects: Aspirin and other salicylates affect many metabolic processes but only at high doses. Aspirin uncouples oxidative phosphorylation in manner similar to 2, 4-dinitrophenol.

In larger doses, it may cause hyperglycaemia and glycosuria and depletes liver and muscle glycogen. Chronic use of large doses causes negative nitrogen balance by enhancing protein catabolism. In suitable doses, salicylates increase urinary excretion of urates, but low doses may decrease urate excretion and elevate plasma urate concentrations. Aspirin reduces plasma free fatty acids and cholesterol levels.

Endocrine effects: High doses of aspirin and other salicylates release epinephrine from the adrenal medulla and very large doses may stimulate ACTH secretion from the anterior pituitary by an action on the hypothalamus.

Pharmacokinetics

Aspirin is easily absorbed from the stomach and proximal small intestine in monogastric animals, although rate of absorption is dependent on factors like stomach contents, gastric emptying and gastric pH. During and after absorption, most of the aspirin is hydrolysed to salicylic acid (salicylate) and acetic acid by esterases present in tissues and blood. Salicylate becomes significantly bound (~70%) to plasma albumin. It is widely distributed in body and penetrates into synovial fluid, peritoneal fluid, saliva and milk, and readily crosses the placental barrier, although diffusion across the blood-brain barrier is limited. Aspirin and other NSAIDs tend to accumulate in inflammatory exudates from which they are cleared more slowly than from plasma.

Aspirin and other salicylates are metabolised in the liver primarily by conjugation with glucuronic acid and glycine, and also by some other metabolic pathways. Some salicylic acid is also excreted unchanged. Neonatal animals (less than 30 days) and cats are less able to metabolise and excrete salicylates. The rate of metabolism is determined by both first order kinetics and zero-order kinetics, depending on the dose used. Excretion of salicylate and its metabolites occur mainly via kidneys by glomerular filtration and renal tubular secretion. Significant tubular reabsorption occurs that is highly pH-dependent i.e. in alkaline pH, tubular reabsorption decreases. Similar to metabolism, the plasma elimination of drug follows first order kinetics at lower doses and zero-order kinetics at higher doses (anti-inflammatory doses). In general, plasma half-life of aspirin in herbivores is about 1 hour, whereas in cats it is about 30 hours. As the elimination of aspirin is dose-dependent, accumulation of toxic levels in plasma and tissue may occur readily with high doses. Alkalisation of urine promotes excretion of the drug.

Side effects/Adverse effects

Therapeutic doses of aspirin cause gastric or intestinal irritation, epigastric distress, nausea, vomiting and/or anorexia. The most important adverse effect of aspirin is gastric mucosal damage and peptic ulceration with varying degree of occult GI blood loss occurring. The buffered aspirin or enteric coated tablet causes less irritation of gastric mucosa than plain uncoated aspirin. High doses may produce hyperventilation, gastric lesions, hepatitis and suppression of erythropoiesis in bone marrow in some cases. Toxic doses cause a centrally mediated hyperpnoea and alkalosis followed by respiratory depression, acidosis, circulatory collapse, hyperpyrexia (due to uncoupling of oxidative phosphorylation), convulsions, coma and death. Death occurs mainly due to respiratory failure. Aspirin is a derivative of phenol, so it is more toxic to cats. Aspirin has shown to be teratogenic in animal studies.

In human beings, repeated high doses of aspirin produce a syndrome called salicylism characterised by dizziness, tinnitus, vertigo, reversible impairment of hearing and vision, excitement, hyperventilation and electrolyte imbalance. Hypersensitivity reactions including rashes, skin eruptions, urticaria, etc. can also be observed in some cases. In humans, aspirin administration has been implicated in the aetiology of 'Reye's syndrome' in infants and children suffering from viral infection characterised by liver damage and encephalopathy. Therefore, aspirin is not given in pyrexia of unknown origin in children below 12 years.

Treatment: Treatment of aspirin overdosage is symptomatic and supportive. Acidosis treatment and forced alkaline diuresis with sodium bicarbonate should be performed for serious ingestion. Most important part is external cooling and IV fluids with Na^+, K^+, HCO_3^- and glucose. Treatment of aspirin induced GI lesions includes the H_2-receptor antagonists (e.g. ranitidine), the PGE_1 analogues (e.g. misoprostol) or the proton pump inhibitor omeprazole.

Contraindications and precautions

Important contraindications of aspirin include cardiac, renal and hepatic diseases, and patients demonstrating hypersensitivity reactions to salicylates. Hypersensitivity to aspirin is a contraindication to therapy with any of the NSAIDs as administration of any one of these could provoke life-threatening reaction resembling an anaphylactic shock. The hypersensitivity reaction does not appear to be immunological in nature but occurs probably due to diversion of arachidonic acid metabolism towards formation of increased amount of leukotrienes and other products of lipoxygenase pathways.

Aspirin should not be used in cases where there is any possibility of gastrointestinal ulceration or bleeding. Aspirin has shown to delay parturition and causes greater post-partum blood loss if given to animals during last stages of pregnancy. Aspirin should be stopped one week before surgery to avoid excessive bleeding. Continuous therapy at inappropriate dosage can lead to early saturation of biotransformation mechanisms, which results in a rapid rise in the amount of drug in body, and the early appearance of toxic symptoms. Because aspirin is highly bound to plasma albumin, patients with hypoalbuminaemia may require lower dosage to prevent symptoms of toxicity. Aspirin should be used cautiously in neonatal animals and cats. Its use should be avoided in dehydrated, hypovolaemic and hypotensive patients.

Drug interactions

Aspirin shows interactions with many drugs. It enhances actions and toxicity of oral anticoagulants and heparin by increasing risk of bleeding. Aspirin displaces warfarin, phenytoin, naproxen, methotrexate, penicillins, sulphonamides, etc. from binding sites on plasma proteins causing adverse reactions and toxicity. It inhibits tubular secretion of uric acid and antagonises uricosuric action of probenecid. It decreases diuretic action of furosemide and thiazides and blocks action of spironolactone. Urinary acidifying drugs like methionine, ammonium chloride and ascorbic acid may decrease the urinary excretion of aspirin. However, drugs which alkalinise the urine (e.g. acetazolamide and sodium bicarbonate) significantly increase the renal excretion of aspirin.

The incidence of GI distress and bleeding is increased when aspirin is used along with steroids and other NSAIDs. Salicylates

have a hypoglycaemic action and potentiate the effects of insulin and sulphonylureas. Phenobarbital may increase the rate of metabolism of aspirin by inducing hepatic enzymes. Gastric lesions and ulceration may be more prevalent in patients given aspirin and other NSAIDs in conjunction with corticosteroids.

Clinical uses

Aspirin is used widely for its analgesic and antipyretic effects in all species. As an analgesic, it is used for the relief of mild to moderate pain of musculo-skeletal origin e.g. myalgia, neuralgia and minor trauma. Aspirin is also used for its anti-inflammatory effect to reduce traumatic and post-surgical oedema and to give relief in conditions like bursitis, arthritis, laminitis and pathogen induced inflammations. Aspirin is used therapeutically for its effects on platelet aggregation in disseminated intravascular coagulation (DIC), thromboembolic disorders and pulmonary artery disease secondary to heartworm infections in dogs. It is used also in cats with cardiomyopathy. In humans, aspirin is used in the prophylaxis of thromboembolic complications like venous emboli and cerebral ischemia associated cardiovascular disorders. Aspirin also reduces risk of transient ischemic attacks in human beings.

Dose

For analgesia and anti-pyrexia

Dogs	:	10 mg/kg, PO, 2 times daily.
Cats	:	10 mg/kg, PO, on alternate days.
Cattle	:	50 – 100 mg/kg, PO, 2 times daily.
Sheep & goats	:	100 mg/kg, PO, 2 times daily.
Horses	:	25 mg/kg, PO, 2 times daily initially then 10 mg/kg, once daily.
Swine	:	10 mg/kg, PO, 4 to 6 times daily.

For anti-inflammatory and anti-rheumatic effects

Dogs	:	25 – 40 mg/kg, PO, 3 times daily.
Cats	:	25 mg/kg, PO, once daily.

For antithrombotic effect/To decrease platelet aggregation

Dogs	:	0.5 mg/kg, PO, 2 times daily.
Cats	:	Up to 75 mg (total dose), PO, every 3 days.

Sodium salicylate

Sodium salicylate is the sodium salt of salicylic acid that is soluble in water and alcohol. It has pharmacological activity similar to that of aspirin but it is about 2/3rd less effective than an equal dose of aspirin. It is used mainly as analgesic, antipyretic and antirheumatic drug. Occasionally, it is used also as a uricosuric drug. It is also considered a potential replacement for aspirin for individuals sensitive to it.

Dose

Dogs	:	10 mg/kg, IV, 2 times daily.
Horses	:	35 mg/kg, IV, 4 times daily.

Diflunisal

Diflunisal is a newer agent and a difluorophenyl derivative of salicylic acid. Its mechanism of action appears to be similar to that of aspirin, but it is not converted to salicylic acid *in vivo*. Diflunisal is more potent than aspirin in anti-inflammatory activity; however, it lacks antipyretic effects perhaps because of poor penetration into the CNS. It is also longer acting than aspirin. The drug has been used primarily as an analgesic in the treatment of osteoarthritis and musculoskeletal strains and sprains in human medicine. Similar to aspirin and other NSAIDs, long-term use of diflunisal leads to an increased risk of stomach ulcers.

Salicylic acid

Salicylic acid is a phenolic phytohormone found in some plants, including the famous willow tree. It is also prepared synthetically and is used in various pharmaceutical preparations. Similar to aspirin, salicylic acid possesses analgesic, antipyretic and anti-inflammatory effects, but it is not used internally due to strong irritating action on the gastric mucosa. It is used only externally on skin mainly in combination with benzoic acid in ointments and alcoholic solutions for antifungal action. Its other important uses are as keratolytic agent to treat acne, warts and other dermatological problems. Various derivatives of salicylic acid have been synthesized for topical and systemic effects. The salts and esters of salicylic acid are often called salicylates.

Other salicylic acid derivatives

Various derivatives or salts of salicylic acid are used in medical and/or veterinary medicine. These include sodium thiosalicylate, choline salicylate, magnesium salicylate, choline magnesium trisalicylate, methyl salicylate (oil of wintergreen), calcium carbaspirin, salsalate (salicylsalicylic acid), phenyl salicylate (salol), benorilate (ester of aspirin and paracetamol) and salicylamide.

2. Pyrazolone Derivatives

Phenylbutazone

Phenylbutazone is a synthetic pyrazolone derivative that was initially introduced into human medicine in 1949 for the treatment of rheumatoid arthritis and other musculoskeletal disorders and subsequent found usage in veterinary species. It is weakly acidic and lipophilic NSAID having analgesic, antipyretic and anti-inflammatory properties but is more toxic than aspirin that limits its use. Phenylbutazone is mainly approved for use in horses and dogs.

Mechanism of action

The mechanism of action of phenylbutazone is similar to that of other NSAIDs in that it inhibits cyclooxygenase activity and hence the synthesis of prostaglandins. It also scavenges toxic oxygen radicals.

Pharmacological effects

Phenylbutazone possesses powerful anti-inflammatory effect (more potent than aspirin) but its analgesic and antipyretic effects are inferior to that of salicylates. It has also mild uricosuric activity, probably attributable to one of its metabolites which decrease tubular absorption of uric acid. Phenylbutazone may reduce renal blood flow, decrease glomerular filtration rate, suppress platelet aggregation and enhance gastric mucosal damage. It causes retention of Na^+, Cl^- and water by direct action on renal tubules; oedema and expansion of plasma volume may occur after few days of use.

Pharmacokinetics

Phenylbutazone is well absorbed orally from both the stomach and small intestine and is approximately 99% bound to plasma proteins. Its high affinity for plasma proteins can displace other highly bound drugs like warfarin. Phenylbutazone is biotransformed primarily in the liver to active metabolites oxyphenbutazone and γ-hydroxyphenylbutazone. Oxyphenbutazone is detectable in the urine for approximately 48 hours

after a single dose of phenylbutazone. Like aspirin, serum half-life of phenylbutazone is dose dependent and ranges between 2.5-6 hours in dogs, 3.5-6 hours in horses, 2-6 hours in swine, 40-55 hours in cattle and 50-65 hours in humans.

Side effects/Adverse effects

In horses, phenylbutazone produces oedema, ulceration of large intestine, diarrhoea, anorexia and necrosis of hepatic vein, especially after prolonged use or high doses. Foals with a heavy parasite burden or those are undernourished and ponies are more susceptible to development of adverse effects. Gastrointestinal ulceration, haemorrhage, biliary stasis and proximal tubule damage have been reported in dogs. Although thought to be less toxic to dogs (due to relatively short elimination half-life), phenylbutazone has been associated with blood dyscrasias in dogs and cats. Manifestations of acute overdosage with phenylbutazone include a prompt acidosis with compensatory hyperventilation, seizures, coma, severe fall in blood pressure and symptoms of renal failure (oliguria, proteinuria and haematuria) and liver injury (hepatomegaly and jaundice). Dose of phenylbutazone only three times greater than the therapeutic dosage have been associated with dilatation and degeneration of the walls of small veins in horses.

Treatment: Phenylbutazone toxicity should be treated with detoxification and supportive therapy. Intravenous diazepam may be used to help control seizures. The use of H_2-blocker, misoprostol (PGE analogue) or sucralfate has been suggested in treating the GI effects.

Contraindications and precautions

Phenylbutazone is contraindicated in patients with a history of or pre-existing haematological or bone marrow abnormalities and pre-existing GI ulcers, renal disease or congestive heart failure. Phenylbutazone should not be used in animals showing hypersensitivity reactions to it. Its use should be avoided in dehydrated, hypovolaemic or hypotensive patients. Phenylbutazone should not be administered by IM or SC route as it is very irritating and may cause sloughing and necrosis of tissues (it may be administered IM in cattle due to less irritation). Regular haematological monitoring is essential in prolonged use. Therapy with phenylbutazone should be stopped at first signs of any toxic reactions. Phenylbutazone should not be used in racehorses for up to 8 days before competition, as it is often misused to enhance performance. Cautions should be exercised when using phenylbutazone in animals less than 6 weeks and aged animals.

Drug interactions

Phenylbutazone and its active metabolite oxyphenbutazone are highly bound to plasma proteins and may displace a number of drugs like warfarin, sulphonamides and phenytoin resulting in their enhanced pharmacological or toxic effects. Phenylbutazone and oxyphenbutazone can induce hepatic microsomal enzymes and increase metabolism of many drugs. Plasma clearance of phenylbutazone may be reduced in animals receiving cimetidine. Concurrent administration of potentially nephrotoxic drugs, aminoglycosides and methoxyflurane anaesthetic should be avoided.

Clinical uses

Phenylbutazone is used mainly in veterinary medicine for the relief of inflammatory and painful conditions associated with the musculo-skeletal system in dogs and horses. It is widely used for the treatment of lameness in horses. Occasionally, it is used as an analgesic and antipyretic in cattle and swine. Its long-half life makes it particularly useful in fractious cattle as dosing can be done at 48-hour intervals. Because of its extensive protein binding and long half-life in cattle, a loading dose of 1.5 to 2 times the maintenance dose is usually employed. Its use in human medicine has declined due to development of blood dyscrasias associated with its use.

Dose

Dogs	:	20 mg/kg, PO, once daily in divided doses for up to 7 days. Then dose is titrated to the lowest effective dose.
Cats	:	25 mg (total dose), PO, 1 to 2 times daily for up to 7 days. Then dose is reduced to 25 mg daily or on alternate days.
Horses	:	4.4 mg/kg, PO, 2 times daily on first day followed by 2.2 mg/kg 2 times daily for 2 to 4 days, then 2.2 mg/kg daily or on alternate days.
	:	2 – 4 mg/kg, slow IV for a maximum of 5 days.
Ponies	:	4.4 mg/kg, PO, once daily for 4 days followed by 2.2 mg/kg, once daily.
Swine	:	4 mg/kg, PO or IV, once daily.
Cattle	:	10 mg/kg, IV or PO priming dose followed by 5 mg/kg, IV or PO maintenance dose every 48 hours.
Sheep & goats	:	1 mg/kg, PO, once daily.

Suxibuzone

Suxibuzone is a prodrug of the non steroidal anti inflammatory drug phenylbutazone. It is commonly used in horses for inflammation, and joint and muscular pain. Gastrointestinal upsets after continuous use or at high dosage are important side/adverse effects. Suxibuzone is generally administered orally with small amount of feed, as its absorption is affected by the presence of hay in GI tract. Its safety in pregnant and lactating animals has not been established. Suxibuzone should be used with caution in animals less than 12 weeks of age.

Dose

Horses	:	12.5 mg/kg, PO in small amount of feed, in 2 divided doses for 2 days, then 6.25 mg/kg daily in 2 divided doses for 3 days, then 3.1 mg/kg daily or on alternate days, then lowest effective dose.
Ponies	:	6.25 mg/kg , PO in small amount of feed, in 2 divided doses for 2 days then 3.1 mg/kg daily or 6.25 mg/kg on alternate days, then lowest effective dose.

Oxyphenbutazone

Oxyphenbutazone is a p-hydroxy analogue of phenylbutazone and one of the active metabolites of phenylbutazone. Oxyphenbutazone has the same spectrum of pharmacological activity, adverse effects, pharmacokinetics, interactions and clinical uses as the parent compound phenylbutazone. It is suggested to have a lesser gastric irritant action than phenylbutazone but is not superior to the parent drug.

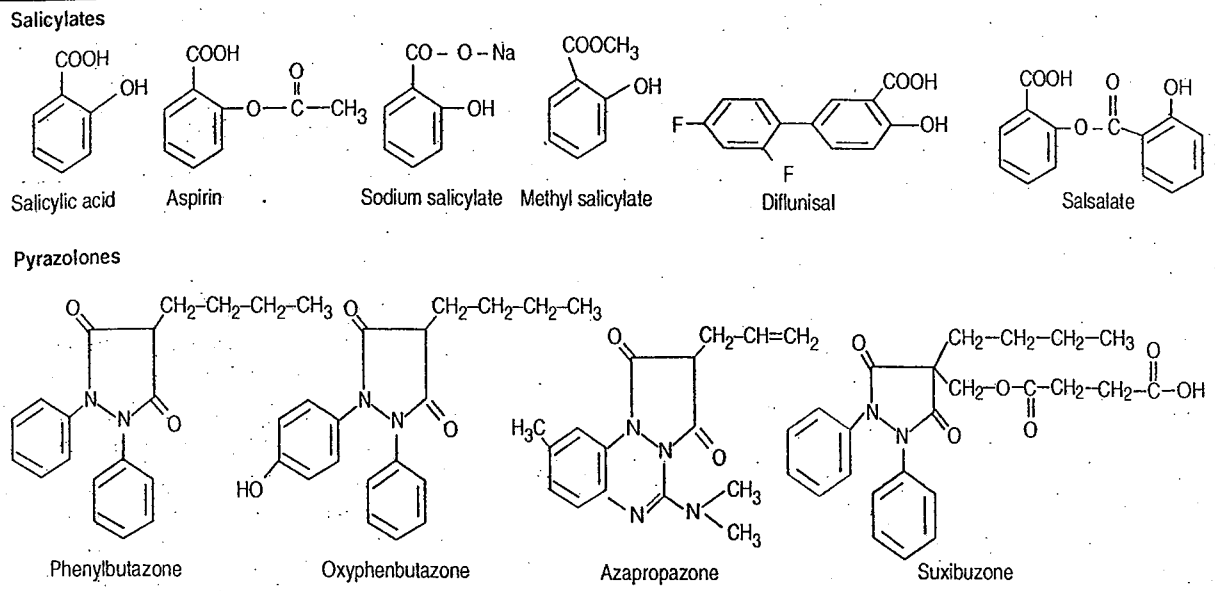


Fig. 30.4. Chemical structures of salicylate and pyrazolone derivative non steroidal anti-inflammatory drugs.

Azapropazone

Azapropazone (Apazone) is a pyrazolone derivative that has anti-inflammatory, analgesic and antipyretic actions but is only a weak inhibitor of cyclooxygenase. In addition, it is a potent uricosuric agent and is particularly useful for the treatment of acute gout. Azapropazone is useful also for the treatment of rheumatic arthritis and osteoarthritis. It has a half life of approximately 20 hours in humans and is not extensively metabolized. Azapropazone possesses high incidence of gastrointestinal side effects and its use in modern medicine has declined.

Other pyrazolone derivatives

Some other pyrazolone derivatives available in market may include phenazone (antipyrine), amidopyrine (aminopyrine) and ramifenazone (isopyrine). Phenazone and amidopyrine are rarely used nowadays in therapeutics due to their bone marrow suppression activity. Ramifenazone is usually used in combination with other NSAIDs, corticosteroids and/or opioid analgesics for the relief of pain and inflammation in soft tissue injury and lameness in animals. Sulfinpyrazone, another pyrazolone derivative, is a powerful uricosuric agent but lacks analgesic and anti-inflammatory activities.

3. Indole and Indene Acetic Acid Derivatives

Etodolac

Etodolac is an indole acetic acid derivative non-steroidal anti-inflammatory drug. In veterinary medicine, etodolac is used mainly in dogs for the management of pain and inflammation associated with osteoarthritis.

Mechanism of action

Like other NSAIDs, etodolac is an inhibitor of cyclooxygenase enzyme. It appears to be more selective for inhibition of COX-2 than COX-1. Therefore, it possesses greater inhibition of the PGs involved with pain and inflammation than those involved with cytoprotection of the GI tract and renal tissue. Etodolac has been shown to inhibit PGE_2 synthesis by macrophages, chondrocytes and synoviocytes. It is also thought to inhibit macrophage chemotaxis, which may explain some of the anti-inflammatory activity.

Pharmacological effects

Etodolac has analgesic, anti-inflammatory and antipyretic effects. It possesses also uricosuric action. Studies in dogs have revealed that etodolac possesses low potential for gastric ulceration in therapeutic doses and has a wide safety margin.

Pharmacokinetics

Etodolac is rapidly and nearly completely absorbed after oral administration in dogs. The peak serum concentration following oral administration occurs between 30 and 60 minutes. Similar to others NSAIDs, etodolac is highly bound to serum proteins. It is metabolized primarily in the liver to various metabolites and is mainly excreted via bile into the faeces. Etodolac undergoes extensive enterohepatic circulation in dogs, which maintains serum concentrations for extended period. The elimination half-life of etodolac in dogs varies from 10 to 14 hours depending whether food is present in the gut, which may affect the rate of enterohepatic circulation of the drug. The half-life in horses is about 3 hours, but duration of effect of approximately 24 hours has been observed in horses with lameness.

Side effects/Adverse effects

Etodolac is well-tolerated drug and side effects occur less frequently than other NSAIDs. However in some dogs, it produces vomiting or regurgitation, diarrhoea, lethargy and hypoproteinaemia. Occasionally, it may cause urticaria, behavioural changes and inappetance. Etodolac has also been associated with keratoconjunctivitis sicca in dogs. In horses, high doses of etodolac cause GI toxicity.

Contraindications and precautions

Etodolac is contraindicated in animals previously found to be hypersensitive to it. It should be used with extreme caution in dogs with pre-existing hepatic, cardiovascular, renal or haemorrhagic abnormalities. It should be used cautiously in patients with severe cardiac failure.

Drug interactions

Etodolac shows interaction with other drugs which are highly protein bound. Concomitant administration of aspirin or other NSAIDs may increase chances of GI adverse effects. Probenecid may cause increase in serum levels and half-life of etodolac.

Clinical uses

Etodolac is used in veterinary medicine mainly for the management of pain and inflammation associated with osteoarthritis in dogs. It may be used to provide post-operative analgesia. It may also be used in other conditions where pain and/or inflammation are involved. Etodolac has been used in some horses to relieve pain associated with abdominal surgery and to treat lameness.

Dose

Dogs : 10 – 15 mg/kg, PO, once daily. For long-term therapy, the dose should be reduced to minimum effective level.

Horses : 20 mg/kg, PO, once daily.

Indometacin

Indometacin (Indomethacin) is a methylated indole derivative, which has prominent anti-inflammatory and analgesic-antipyretic properties similar to those of salicylates. Indometacin has analgesic properties independent of its anti-inflammatory action, and there is evidence for both peripheral and central effects. Indometacin is a potent inhibitor of PGs, especially PGE_2, and blocks the motility of polymorphonuclear leucocytes. It also uncouples oxidative phosphorylation in cartilaginous and hepatic mitochondria at high dosages. Indometacin has very short elimination half-life (0.3 hours) in dogs. Indometacin is not used in dogs because it is more ulcerogenic than other NSAIDs in this species. It also has limited use in other veterinary species and is not considered a preferred NSAID in veterinary medicine. Indometacin is commonly used in human beings to reduce fever, pain, stiffness and swelling. In human medicine, indometacin is also considered an effective tocolytic agent, which delays premature labour by reducing uterine contractions through inhibition of PG synthesis in the uterus and possibly through calcium channel blockade.

Sulindac

Sulindac is closely related to indometacin and has analgesic-antipyretic and anti-inflammatory activity. Sulindac is a pro-drug that metabolises to its active sulphide metabolite. The sulphide metabolite is about 500 times more potent than sulindac as an inhibitor of cyclooxygenase enzyme. Sulindac has been used mainly for treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis in man but is not approved for use in animals.

4. Oxicam Derivatives

Piroxicam

Piroxicam is a novel oxicam derivative NSAID with anti-inflammatory, analgesic and antipyretic activities. It occurs as white crystalline solid that is sparingly soluble in water. Piroxicam is structurally not related to other non-steroidal anti-inflammatory drugs.

Mechanism of action

Like most other NSAIDs, the primary action of piroxicam is thought to be due to the reversible inhibition of cyclooxygenase enzyme. It lowers prostaglandins concentration in synovial fluid and inhibits platelet aggregation. Piroxicam is thought to have relatively high COX-2 (IC_{50}): COX-1 (IC_{50}) inhibition ratio (Table 30.1). Additional mechanisms involved in its anti-inflammatory action include inhibition of superoxide formation, inhibition of activation of neutrophils, and inhibition of proteoglycanase and collagenase in cartilages. In addition, it decreases production of IgM rheumatoid factor.

Pharmacological effects

Piroxicam possesses prominent anti-inflammatory and good analgesic-antipyretic activity. In recommended doses, its anti-inflammatory potency is equivalent to aspirin. Like other NSAIDs, it inhibits platelet aggregation and prolongs bleeding time, causes GI mucosal damage and can affect renal function. Piroxicam may also have some antitumour or tumour preventive effects and is used in some anticancer protocols.

Pharmacokinetics

Piroxicam is well absorbed after oral administration. Although presence of food in GI tract may decrease the rate of absorption, the total amount of piroxicam absorbed remains same. Piroxicam is highly bound to plasma proteins. It is largely metabolised in liver by hydroxylation and glucuronide conjugation, and is excreted in urine and bile. It is excreted also in milk in very low concentrations (about 1%). Piroxicam undergoes entero-hepatic cycling. In dogs, piroxicam has a very long plasma half-life (about 40 hours) as result of which steady state concentrations are achieved in a week.

Side effects/Adverse effects

Adverse GI reactions are most common harmful effects of piroxicam. Serious GI toxicity in the form of bleeding, peptic ulceration and perforation has been reported in dogs after long-term use of the drug. In general, piroxicam is comparatively less ulcerogenic than phenylbutazone and causes less faecal blood loss than aspirin. Other adverse effects may include renal papillary necrosis, peritonitis, CNS effects, tinnitus, oedema, pruritus and rash, and elevation in hepatic function tests. Patients ingesting significant overdosage should be monitored carefully.

Treatment: Treatment of piroxicam over dosage is symptomatic and supportive.

Contraindications and precautions

Piroxicam should not be used in patients having pre-existing or history of GI ulcers or bleeding disorders. It is contraindicated in patients hypersensitive to it or other NSAIDs. It should be used with extreme caution in patients with congestive heart failure.

Drug interactions

The use of piroxicam in conjunction with aspirin or other NSAIDs may increase the potential of adverse effects. Because piroxicam is highly bound to plasma proteins, it can displace or get displaced by other highly protein bound drugs like warfarin and phenylbutazone.

Clinical uses

In veterinary medicine, piroxicam is used mainly in dogs for reducing the pain and inflammation associated with degenerative joint disease (e.g. osteoarthritis). It is also used in veterinary medicine in some anticancer protocols to treat or suppress certain neoplasias expressing cyclooxygenase (COX) receptors, such as transitional cell carcinoma of bladder, squamous cell carcinoma

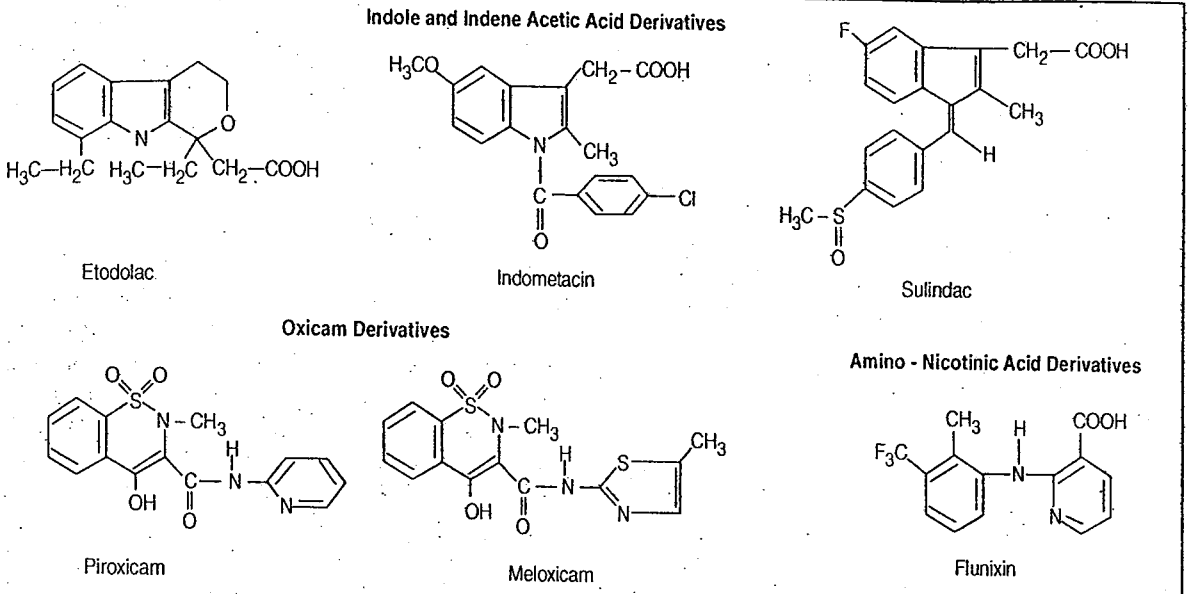


Fig 30.5. Chemical structures of selected indole and indene acetic acid, oxicam and aminonicotinic acid derivative non-steriodal anti-inflammatory drugs.

and mammary adenocarcinoma. It is also used in combination with cisplatin to treat oral malignant melanoma and oral squamous cell carcinoma in dogs. In humans, it is used for the treatment of rheumatoid arthritis and osteoarthritis, and acute musculoskeletal disorders, dysmenorrhoea, post-operative pain and acute gout.

Dose

For analgesic/anti-inflammatory effects (osteoarthritis)

Dogs : 0.3 mg/kg, PO, on alternate days.

As an adjunctive therapy of transitional cell carcinoma

Dogs & cats : 0.3 mg/kg, PO, once daily with food.

Meloxicam

Meloxicam is a recently developed congener of piroxicam that exhibits anti-inflammatory, antipyretic and analgesic activities. Meloxicam has greater *in vitro* and *in vivo* inhibitory action against the inducible COX-2, which is implicated in the inflammatory response than COX-1. In animal studies, a COX-2: COX-1 selectivity of 11-14 times has been measured. Accordingly, the anti-inflammatory efficacy of meloxicam has been found to be similar to piroxicam, while GI and renal side effects are significantly less. The elimination half-life of meloxicam is reported to be shorter in horses (~3 hours) and pigs (4 hours), while it is longer in dogs (12-36 hours), cattle (~13 hours) and man (20 hours). Oral absorption is almost complete in dogs when administered with food.

In veterinary medicine, meloxicam is used mainly in dogs for relieving pain and inflammation associated with osteoarthritis and synovitis. It is also used as an antipyretic in dogs and cats. In pigs, meloxicam has been recommended for the treatment of mastitis-metritis-agalctia syndrome. In horses, meloxicam is used for treating pain and inflammation associated with surgery. It is also used for treatment of some other conditions such as mastitis in cattle and diarrhoea in calves. Meloxicam has fewer gastrointestinal side effects than piroxicam and perhaps other NSAIDs which are not COX-2 selective. However, similar to other NSAIDs, gastrointestinal toxicity (inappetance, vomiting and diarrhoea), and bleeding may occur after prolonged use or in high doses. Meloxicam use should be avoided for treatment of diarrhoea in calves less than one week of age and in cats less than 2 kg body-weight. Meloxicam is mostly given by addition to food or soon after feeding.

Dose

Dogs : 0.2 mg/kg, PO, SC or IV, as a single dose for 1 day, then 0.1 mg/kg, once daily.

Cats : 0.2 – 0.3 mg/kg, SC or PO as a single dose for 1 day, then 0.1 mg/kg, PO, 2 times weekly.

Pigs : 0.4 mg/kg, IM, once daily.

Horses : 0.6 mg/kg, PO or IV, once daily.

Birds : 0.5 mg/kg, PO or SC, once daily.

Other oxicam derivatives

Some other oxicam derivatives are under study in animals or are presently used in human medicine. These include cinnoxicam, droxicam, lornoxicam (chlortenoxicam), tenoxicam and sudoxicam. Ampiroxicam is a pro-drug of piroxicam; it is well absorbed when given orally but achieves maximal plasma concentrations later than does piroxicam.

5. Amino-Nicotinic Acid Derivatives

Flunixin

Flunixin meglumine is a nicotinic acid derivative, which has prominent anti-inflammatory, analgesic and antipyretic activities. It is very potent inhibitor of cyclooxygenase and is used mainly in veterinary medicine in horses and also some other species like cattle and swine.

Pharmacological effects

Like other NSAIDs, flunixin exhibits analgesic, anti-inflammatory and antipyretic effects. As an analgesic, flunixin is more potent than phenylbutazone, pethidine and pentazocine. It is also four times as potent as anti-inflammatory than phenylbutazone in horses. It significantly alleviates pain arising from spasms (colic) of the GI tract and is particularly useful for

control of severe visceral pain. Flunixin does not appreciably alter GI motility in horses and may improve haemodynamic in animals with septic shock.

Pharmacokinetics

Flunixin is absorbed up to 80% following oral administration in horses. Peak serum concentration is attained in 30 minutes, peak response occurs in 12-16 hours and duration of action lasts for 24-36 hours. The serum half-life of flunixin has been reported to be about 1.5-2 hours in horses, 3.5 hours in dogs and 8 hours in cattle. Despite its short half-life in horses, its duration of action is longer (>24 hours) and it can be detected in urine for at least 48 hours after a single dose. This occurs probably due to binding of flunixin to hay in the GI tract of horses (also ruminants) from where it is slowly and continuously released, metabolised and excreted from body.

Side effects/Adverse effects

Flunixin appears to be a relatively safe agent for use in horses. Occasionally, it produces GI intolerance, hypoproteinaemia and haematological abnormalities. Intramuscular injection may produce localised swelling, indurations, stiffness and sweating. In dogs, flunixin may produce GI distress, vomiting, diarrhoea and ulceration with high doses or chronic use. In horses and cattle, rare anaphylactic-like reactions have been reported, mainly after rapid IV administration.

Treatment: Flunixin overdosage should be treated symptomatically and supportively.

Contraindications and precautions

Flunixin should not be used in animals with a history of hypersensitivity reaction to it or aspirin. It should be used with caution in animals with pre-existing GI ulcers, renal, hepatic or haematological disorders, and in young (< 6 weeks of age) and old animals. Repeated use of flunixin in equine colic is generally not recommended due to the pain-masking effect of drug (drug may obscure diagnosis). Intra-arterial injection should be avoided as flunixin causes transient signs of excitement and muscle weakness. Flunixin is considered to be contraindicated in cats, but some veterinarians use it for short-term therapy. It should not be used in animals for human consumption.

Drug interactions

Drug interactions have not been fully studied for flunixin, but it may follow other NSAIDs. Co-administration with enrofloxacin in dogs increases flunixin plasma concentration because of reduced clearance.

Clinical uses

Flunixin meglumine is used widely for the alleviation of inflammation and pain associated with musculo-skeletal disorders, and alleviation of visceral pain associated with colic in horses. In colic in horses, the analgesic potency of flunixin provides temporary improvement even in cases for which surgery would be essential, providing time for preparation and examination. Other uses in horses may include shock, colitis, post-race treatment and pre- and post-ophthalmic and general surgery. In cattle, it is approved for control of pyrexia and inflammation with bovine respiratory diseases and endotoxaemia. It may also be used in bovine mastitis. In dogs, flunixin is used for arthritis, diarrhoea, shock, ophthalmic and other inflammatory conditions, and treatment of parvovirus infection. It is also effective in injuries of the eye. It may be indicated for pain, shock and trauma in birds.

Flunixin may be given orally as a paste or as granules in feed. It can also be used intramuscularly, subcutaneously or intravenously, but due to its irritant nature to tissues the IV route is preferred.

Dose

For anti-inflammatory and analgesic effects (musculo-skeletal disorders)

Dogs	:	1 mg/kg, PO, once daily for up to 3 days. Three-day course may be repeated once weekly for chronic conditions.
Cats	:	0.25 mg/kg, SC or IM, once only; may be repeated in 12-24 hours, if needed.
Horses	:	1.1 mg/kg, PO, IM or IV, once daily for up to 5 days.
Cattle	:	2.2 mg/kg, slow IV, once daily for up to 5 days.
Swine	:	1.1 – 2.2 mg/kg, IV, IM, PO, 1 to 2 times daily or as needed based on clinical response.
Sheep & goats	:	1 mg, IV, once daily.
Birds	:	1 – 10 mg/kg, IM.

For pyrexia

Dogs & cats	:	0.25 mg/kg, IV, SC or IM, once daily; may be repeated in 12-24 hours, if needed.

For equine colic

Horses	:	1.1 mg/kg, IV; may be repeated 1-2 times.

For endotoxic shock

Dogs	:	1 mg/kg, slow IV, up to 2 times daily for maximum of 3 doses.
Horses	:	0.25 mg/kg, slow IV, 3 to 4 times daily.

For eye use

Dogs	:	0.5 mg/kg, IV, single use.

Clonixin

Clonixin is an amino-nicotinic acid derivatives non-steroidal anti-inflammatory drug. It possesses analgesic, antipyretic and platelet-inhibitory actions. It is used primarily in human medicine in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation.

6. Hetero-Aryl Acetic Acid Derivatives

Diclofenac

Diclofenac sodium is a phenylacetic acid that possesses analgesic, antipyretic and anti-inflammatory activities. It is primarily an inhibitor of cyclooxygenase enzyme system and its potency is greater than that of naproxen and several other NSAIDs. On cyclooxygenase system, diclofenac appears to have a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) and is said to have therefore a somewhat lower incidence of gastrointestinal problems than noted with aspirin and other NSAIDs. Other modes of action include inhibition of DNA synthesis, reduction of neutrophil chemotaxis and superoxide production at the inflammatory site, blockade of voltage-dependent sodium channels and some inhibitory action on lipoxygenase pathways. There is also speculation that diclofenac may inhibit phospholipase A_2 as part of its mechanism of action. These additional actions make the diclofenac a highly potent NSAID.

Diclofenac is used mainly in human medicine in rheumatoid and osteoarthritis, bursitis, spondylitis, polymyositis, dysmenorrhoea, dental pain, and post-traumatic and post-operative

inflammatory conditions. It is also considered useful in the pain management of kidney stones and gallstones. Diclofenac is occasionally also used to treat chronic pain associated with cancer, particularly if inflammation is also present. Diclofenac is also an antiuricosuric drug. In veterinary medicine, diclofenac sodium is used mainly for treatment of uveitis, especially when corneal infection is suspected. It is also used occasionally for alleviation of pain and inflammation in animals. In humans, diclofenac is better tolerated than most commonly NSAIDs. However, GI upsets may occur with its regular use and development of ulceration and/or bleeding requires immediate termination of treatment with diclofenac. Diclofenac is also associated with adverse renal effects caused by the reduction in synthesis of renal prostaglandins in sensitive persons or animal species.

The veterinary use of diclofenac has earned a notoriety of causing wide spread deaths of vultures in Indian subcontinent. Diclofenac is highly toxic to vultures and the mechanism is renal failure, a known side-effect of diclofenac. Vultures eat the carcasses of livestock which have been administered veterinary diclofenac and are poisoned by the accumulated drug. Meloxicam has been recommended to replace use of diclofenac in veterinary species, so as to prevent extinction of vultures.

Dose

Diclofenac sodium (0.1% ophthalmic solution)

All species : One drop every 30 minutes starting 2 hours before surgery for a total of 4 drops.
: One drop four times daily following cataract surgery or for the treatment of uveitis.

Ketorolac

Ketorolac tromethamine is a NSAID in the family of heterocyclic acetic acid derivative, often used as an analgesic, antipyretic and anti-inflammatory. It inhibits PGs synthesis and is believed to relieve pain by a peripheral mechanism. Like most NSAIDs, ketorolac is a non-selective COX inhibitor. Ketorolac is a potent analgesic and its efficacy in post-operative pain has been considered almost equal to that of morphine. Unlike opioid analgesics, it is not associated with tolerance or respiratory depression. Ketorolac also has anti-inflammatory activity when topically administered in the eye.

In veterinary medicine, ketorolac is primarily used to control surgical or non-surgical uveitis, particularly in cases with concurrent corneal infection when topical corticosteroids are contraindicated or in diabetic patients. In human medicine, ketorolac is used for post-operative pain, as an alternative to opioid agonists and for acute musculo-skeletal pain. In addition, it is used for inflammatory conditions in the eye and for treatment of seasonal allergic conjunctivitis.

Dose

Ketorolac tromethamine ophthalmic (0.5% solution)

All species : One drop, 4 times at 20 minute intervals, before surgery.
: One drop, 4 times daily following cataract surgery.
: One drop, four times daily for treatment of uveitis or for management of allergic conjunctivitis.

For surgical pain

Dogs : 0.3 – 0.5 mg/kg, IM or IV, every 8-12 hours for one to two treatments.

Cats : 0.25 mg/kg, IM, every 12 hours for one to two treatments.

Goats : 0.3 – 0.7 mg/kg, IV, IM, SC, PO, 3 times daily.

Eltenac

Eltenac is a NSAID that is used primarily in veterinary medicine as an anti-inflammatory and analgesic agent. It is used also in equine colic. Its repeated use in equine colic is not generally recommended due to pain masking effect of the drug. Prolonged use may cause gastro-intestinal lesions, inappetance, vomiting and diarrhoea.

Dose

Horses : 0.5 mg/kg, slow IV, once daily for 5 days.

Aceclofenac

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) used mainly for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. In addition to blocking the cyclooxygenase enzyme, aceclofenac is an inhibitor of cytokines. Aceclofenac has higher anti-inflammatory action than conventional NSAIDS.

Tolmetin

Tolmetin is an effective anti-inflammatory agent with analgesic and antipyretic effects. In recommended doses, it appears to be approximately equivalent in efficacy to moderate doses of aspirin. It is used primarily in human medicine for osteoarthritis and rheumatoid arthritis.

II. AGENTS WITH ANALGESIC AND MILD TO MODERATE ANTI-INFLAMMATORY ACTIVITY

1. Propionic Acid Derivatives/Aryl Propionic Acid Derivatives

Propionic acid derivatives are an important and useful group of non-steroidal anti-inflammatory drugs. They may offer significant advantages over aspirin and are better tolerated. Naproxen, the prototype propionic acid NSAID, is used in both human and veterinary medicines, whereas carprofen, ketoprofen and vedaprofen have been developed for veterinary use only.

Naproxen

Naproxen is a propionic acid derivative non-steroidal anti-inflammatory drug. It is practically insoluble in water and freely soluble in alcohol. Naproxen is a non-selective (COX-1 and COX-2) NSAID and mediates its action through inhibition of prostaglandins synthesis. Additionally, naproxen inhibits leucocytes migration.

Pharmacological effects

Like other NSAIDs, naproxen exhibits analgesic, anti-inflammatory and antipyretic activities. Some studies have shown that naproxen also possesses a cardioprotective effect compared to other NSAIDs.

Pharmacokinetics

The pharmacokinetics of naproxen varies between species. Bioavailability after oral administration is about 50% in horses and 70-100% in dogs. However, absorption does not appear to be altered by the presence of food. Like most NSAIDs, it is highly bound to plasma proteins. The elimination half-life of naproxen in different species is: horses = 20 hours, cattle = 13 hours, dogs = 35-74 hours and man = 20 hours. In dogs, there are apparently breed differences in half-life; half-life is reported

to be 72 hours in Mongrels and 35 hours in Beagles. This long half-life is likely due to enterohepatic cycling in the dogs.

Side effects/Adverse effects

Adverse effects of naproxen are apparently uncommon in horses because it has relatively wide margin of safety in this species. Occasionally, gastrointestinal (diarrhoea and ulcers), renal (fluid retention), haematological (decreased haematocrit) and CNS (neuropathies) effects may occur in horses. Naproxen is believed to be more toxic in dogs because of apparent narrow therapeutic index, prolonged half-life and seriousness of the potential adverse reactions. Dogs may be overly sensitive to the adverse GI (ulcers and perforation), renal (nephritis) and hepatic (hepatitis) effects. Fatalities due to naproxen have been reported in dogs receiving the drug at equivalent human or equine dosages.

Treatment: Naproxen overdosage should be treated supportively and symptomatically. Because naproxen may cause renal effects, careful monitoring of electrolyte and fluid balance is advised.

Contraindications and precautions

Contraindications and precautions of naproxen are similar to those of carprofen. Caution is advised when using the human formulations in dogs because the human tablet size is much larger than safe doses for dogs.

Drug interactions

Drug interactions are similar to those of carprofen. Probenecid may cause a significant increase in serum levels and half-life of naproxen. Corticosteroids have been shown to exacerbate the GI adverse effects of naproxen.

Clinical uses

In veterinary medicine, naproxen is used primarily in horses for the relief of inflammation and associated pain and lameness exhibited by diseases of musculo-skeletal system. It has also been used as an anti-inflammatory and analgesic in dogs for the treatment of osteoarthritis and other musculo-skeletal inflammatory diseases. In humans, it is recommended for the reduction of pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, rheumatoid arthritis, gout, ankylosing spondylitis, menstrual cramps, tendinitis and bursitis.

Dose

Horses	:	10 mg/kg, PO, 2 times daily for up to 14 days.
	:	5 mg/kg, slow IV, followed by 10 mg/kg, PO 2 times daily for up to 14 days.
Dogs	:	5 mg/kg, PO, initially, then 2 mg/kg, on alternate days.

Carprofen

Carprofen is a newly developed propionic acid derivative NSAID for use in animals. It is practically insoluble in water and freely soluble in ethanol. Carprofen exists in two enantiomeric forms: R(–) and S(+), and is formulated as racemic (50: 50) mixture of these enantiomers.

Mechanism of action

The principal mechanism of action appears to involve preferentially selective inhibition of COX-2 enzyme, with less interference with activity of COX-1 enzyme. Therefore, physiological or protective actions of prostaglandins appear to be minimally inhibited with no loss of anti-inflammatory efficacy. Carprofen has also been demonstrated to modify cell-mediated immune responses. Other proposed mechanisms for carprofen include inhibition of phospholipase enzyme and impaired release of arachidonic acid.

Pharmacological effects

Similar to several other NSAIDs, carprofen exhibits analgesic, anti-inflammatory and antipyretic effects. It seems to provide pain control comparable and even better than that achieved with some opioid analgesics.

Pharmacokinetics

Carprofen is well absorbed (~ 90%) following oral administration and its peak serum levels occur between 1-3 hours post-dosing in dogs. It is highly bound to plasma proteins (99%) and has a low volume of distribution. Carprofen is metabolised mainly by oxidation and glucuronide conjugation in liver and is excreted in faeces (70-80%) and urine (10-20%). Carprofen also undergoes some enterohepatic cycling in dogs, which may prolong the duration of action. Elimination half-life of carprofen in different species is: dogs = 8-15 hours, horses = 22 hours, cats = 15-20 hours and man = 1 - 2 hours.

Side effects/Adverse effects

Carprofen has fewer side effects than other NSAIDs because of its weak antagonistic action on cyclooxygenase-1 (COX-1) enzyme. Mild gastrointestinal effects are the most likely side effects. Rarely hepatocellular damage, renal disease, and haematological and serious gastrointestinal effects may occur. Labrador Retrievers have been associated with 1/3 of the initial cases associated with the reported hepatic syndrome. In dog toxicological studies, repeated doses of up to 10 times resulted in little adverse effects. Excess use of carprofen can lead to gastritis and ulcer formation.

Contraindications and precautions

Carprofen is contraindicated in dogs with bleeding disorders, GI ulcers and cardiac or hepatic diseases. Carprofen should not be used in patients hypersensitive to it or other propionic class NSAIDs. It should be used with caution in geriatric and young patients and pregnant animals. Its use should be avoided in dehydrated, hypovolaemic and hypotensive patients. Carprofen has produced toxicity in cats if administered at the same dose rates as for dogs.

Drug interactions

Carprofen should not be used along with nephrotoxic drugs. Concurrent use of aspirin or other potent NSAIDs may enhance ulcerogenic effect of carprofen. Like other NSAIDs, it shows displacement drug interactions.

Clinical uses

Carprofen is mainly used for the relief of pain and inflammation in dogs and other species. It is used also for the treatment of osteoarthritis in dogs. For control of surgical pain, carprofen may be administered before the patient recovers from anaesthesia (NSAIDs which are potent cyclooxygenase inhibitors are not used before patient regains consciousness after general anaesthesia because they can occasionally precipitate acute renal failure, notably in the dogs). Carprofen has also been used to reduce morbidity and mortality in calf pneumonia by suppressing oedema. Carprofen is no longer marketed for human usage, after being withdrawn on commercial grounds.

Dose

Dogs	:	2 – 4 mg/kg/day, PO, in divided doses for 7 days, then 2 mg/kg once daily.

Dogs & cats : 4 mg/kg, SC or IV as a single dose.
Cats : 0.5 mg/kg, PO, once daily for long-term use.
Horses : 0.7 mg/kg, PO, once daily for up to 4 to 9 days.
: 0.7 mg/kg, IV as a single dose. Dose may be repeated after 24 hours, if required.
Cattle : 1.4 mg/kg, SC or IV as a single dose.
Swine : 2 – 4 mg/kg, IV, once daily.

Ketoprofen

Ketoprofen is an arylpropionic acid that is used in veterinary medicine mainly for use in dogs, cats and horses. It is formulated and used as a racemic (50: 50) mixture of R(-) and S(+). Like other NSAIDs, ketoprofen possesses analgesic, anti-inflammatory and antipyretic activities. Its primary mechanism of action is non-selective inhibition of cyclooxygenase enzyme, thereby inhibition of the synthesis of PGs. In addition, it is said to stabilise lysosomal membranes and may antagonise action of bradykinin. It is also reported to possess lipoxygenase inhibitory action, but the action is small enough to be of any clinical significance.

Similar to other propionic acid derivatives, ketoprofen is rapidly and nearly completely absorbed after oral administration and is highly plasma protein bound (~ 93% in horses). The presence of food decreases the rate but not extent of absorption. In horses, the onset of action occurs within 2 hours and peak effect is seen at about 12 hours of dosing. The elimination half-life of ketoprofen in different species is: dogs = 3.5 hours, cats = 1.6 hours, horses =1.5 hours and cattle = 0.4 hours. It is eliminated via the kidneys both as conjugated metabolite and unchanged drug.

Ketoprofen appears to have a lower incidence of adverse effects than aspirin, phenylbutazone or flunixin. Occasional transient vomiting, diarrhoea gastric mucosal damage, GI ulceration, renal crest necrosis and mild hepatitis may occur. In horses, the drug is considered to be less ulcerogenic and nephrogenic than other common NSAIDs. Very high doses of ketoprofen may result in anorexia, depression and laminitis in horses, especially when given for few days. Its use should be avoided in racehorses 15 days prior to racing, in pregnant animals and before regaining consciousness after any general anaesthesia. Drug interactions and contraindications are similar to other NSAIDs. Ketoprofen is used mainly for alleviation of inflammation and pain associated with musculo-skeletal disorders. In horses, it is also used for control of abdominal pain and other inflammatory conditions. In cattle, it has been found effective for fever, pain and inflammation associated with mastitis. Ketoprofen in small animals is mostly used by injection for acute conditions and by tablet for long-term use.

Dose

Dogs & cats : 2 mg/kg, SC, IM or IV, once daily for up to 3 days, followed by 1 mg/kg, once daily.
: 1 mg/kg, PO, once daily for up to 5 days.
Horses : 2.2 – 3.3 mg/kg, IV or IM, once daily for 3 to 5 days.
Cattle : 3 mg/kg, IM or IV, once daily for up to 3 days.
Swine : 3 mg/kg, IM as a single dose.

Vedaprofen

Vedaprofen is another propionic acid derivative that has been developed for use in veterinary medicine. Like carprofen, it exists in two enantiomeric forms and is formulated as racemic (50: 50) mixture of R(-) and S(+) forms. Both enantiomers contribute to the biological and therapeutic actions of vedaprofen. It possesses analgesic, antipyretic and anti-inflammatory activities based on inhibition of prostaglandins synthesis.

Vedaprofen is rapidly and almost completely (80-90%) absorbed after oral administration. It is highly bound (>99%) to plasma proteins. It is metabolized in liver to inactive/less active metabolites and excreted mainly in urine (70%) and faeces (10-15%). The elimination half-life of vedaprofen in horses is about 6-8 hours. Vedaprofen is generally well tolerated in recommended doses. Important side effects are GI upsets with ulcerogenic effects. It is primarily used in horses and dogs. In horses, it is recommended for control of pain and inflammation associated with musculo-skeletal disorders and trauma, and soft tissue lesions.

Dose

Dogs : 0.5 mg/kg, PO, once daily for 28 days maximum.
Horses : 2 mg/kg, PO, followed by 1 mg/kg, PO, 2 times daily for 14 days.

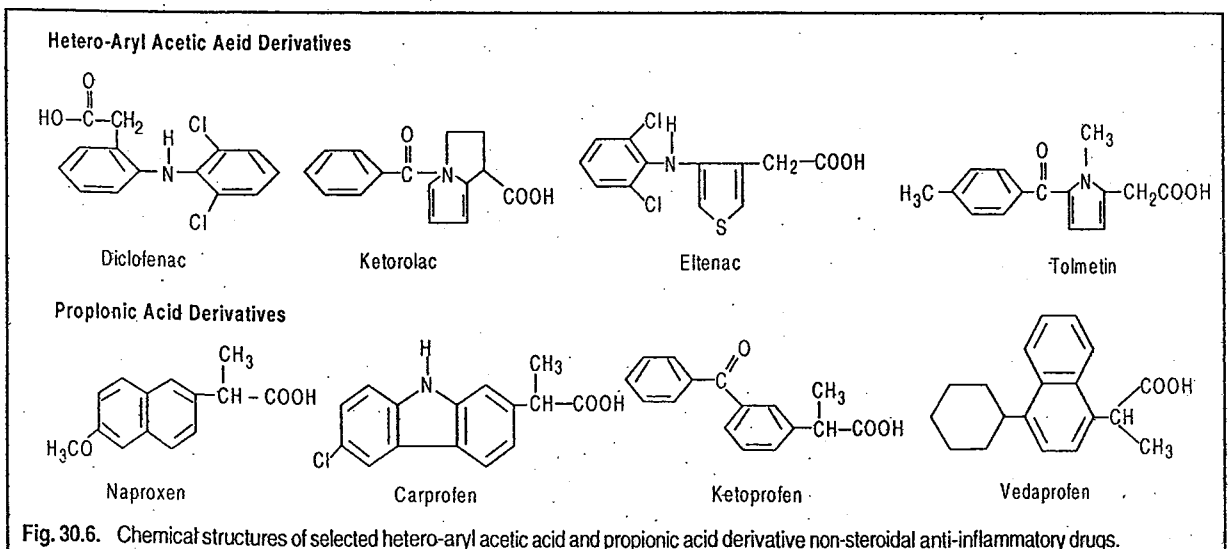


Fig. 30.6. Chemical structures of selected hetero-aryl acetic acid and propionic acid derivative non-steroidal anti-inflammatory drugs.

Ibuprofen

Ibuprofen was the first member of propionic acid derivatives class to be introduced in therapeutics in 1969. In humans, it is an effective anti-inflammatory and analgesic agent, and is associated with relatively low level of GI toxicity. Unfortunately, similar safety has not been associated with ibuprofen use in dogs. It has a narrow margin of safety for use in dogs and cats, such that repeated doses of more than 5 mg/kg, daily can result in intoxication in dogs. Overdose or intoxication gives rise to gastrointestinal irritation with enteritis, haemorrhage and possible perforation. It also produces renal failure and acidosis in dogs. In human medicine ibuprofen is used primarily for arthritis, fever, and control of pain, especially where there is an inflammatory component.

Other propionic acid derivatives

Several other propionic acid derivatives are available but are mainly used in human medicine. These include fenoprofen, flurbiprofen and oxaprozin (oxaprozinum). Flurbiprofen is considered to be one of the potent inhibitors of prostaglandins synthesis and is found to be more potent in this respect than aspirin, indometacin, piroxicam, diclofenac, naproxen and ibuprofen.

Dose

Fenoprofen

Dogs : 0.5 – 1 mg/kg, PO.

2. Anthranilic Acid Derivatives/Fenamates

Meclofenamic acid

Meclofenamic acid (Meclofenamate) is an anthranilic acid derivative that possesses analgesic, antipyretic and anti-inflammatory activity. Meclofenamic acid is practically insoluble in water but its sodium salt is commercially available that is freely soluble in water. It is a potent inhibitor of cyclooxygenase enzyme, mainly COX-2, thereby inhibiting synthesis of prostaglandins and related compounds. In high concentrations, it may have antagonistic effects on histamine and kinins.

Pharmacological effects

Meclofenamic acid exhibits pharmacological actions similar to aspirin. It has analgesic, antipyretic and anti-inflammatory actions. As an analgesic, it is more efficacious than phenylbutazone. Intravenous injection of meclofenamic acid has been demonstrated to reduce the cardiovascular and respiratory effects of experimentally induced anaphylaxis in ponies and calves. Moreover, it antagonises effects of bradykinin and PGs on bovine pulmonary smooth muscle. It also has a transient effect on platelet aggregation, but unlike aspirin it does not appear to affect bleeding time.

Pharmacokinetics

Meclofenamic acid is well absorbed after oral administration with peak plasma levels being reached in 1-4 hours. It is highly bound (>98%) to plasma proteins. It is metabolised in liver primarily by oxidation to an active hydroxymethyl metabolite, which may be further oxidised to an inactive metabolite. Meclofenamic acid and its metabolites are excreted both in urine (mainly) and faeces. The plasma half-life has been reported to range from 6 to 8 hours in horses. The therapeutic action of meclofenamic acid does not seem to be closely related with blood levels or plasma half-life as onset of action may take 36-96 hours and significant effect may be seen for few days following a single dose. In horses, meclofenamic acid can be detected in urine for at least 4 days following the final dose. The prolonged action of meclofenamic acid makes daily dosing satisfactory.

Side effects/Adverse effects

Side effects of meclofenamic acid are related to aspirin, but are fairly uncommon in horses. Gastrointestinal effects (diarrhoea, colic, mucosal erosions and anorexia) and haematological changes (decreased haematocrit) have been reported in horses. With chronic therapy, decrease in plasma protein concentration may occur. In dogs, important adverse effects include vomiting, tarry tools and decreased haemoglobin and small intestine ulcers. Meclofenamic acid and other fenamates have been associated with a relatively high incidence of gastrointestinal toxicity (diarrhoea, vomiting and other GI disorders) and hepatotoxicity in humans. Generalised CNS stimulation, seizures and acute renal failure has been associated with massive overdoses in humans.

Treatment: Treatment of meclofenamic acid overdosage is supportive and symptomatic.

Contraindications and precautions

Important contraindication of meclofenamic acid includes GI, hepatic or renal diseases and patients demonstrating previous hypersensitivity to it or salicylates. It has been shown to delay parturition in some species and, therefore, should be avoided during the last stages of pregnancy. Meclofenamic acid has caused teratogenic effects in experimental animals, so it should be used in pregnancy only when the potential benefits outweigh the potential risks of therapy. Meclofenamic acid should not be used in lactating animals, in breeding animals within 5 days of administration of prostaglandins and in racehorses 8 days prior to racing.

Drug interactions

Concomitant use of aspirin with meclofenamic acid is not advised because of increased GI adverse effects. Similar to other NSAIDs, it shows drug displacement interactions.

Clinical uses

Meclofenamic acid is used in horses and dogs for the symptomatic relief of acute or chronic inflammatory diseases involving the musculo-skeletal system. It has been used in horses for the treatment of laminitis, with varying degree of success. Meclofenamic acid is marketed as granule formulation for use in horses.

Dose

Horses : 2.2 mg/kg, PO, once daily for 5 to 7 days best in feed. If treatment is required past 7 days, dosage may be reduced and interval may be increased to lowest effective level.

Dogs : 1.1 mg/kg, PO, once daily for 5 to 7 days. Dosage may be reduced, if required past 7 days.

Cats : 2.2 mg/kg, PO, once daily.

Tolfenamic acid

Tolfenamic acid is similar in activity to meclofenamic acid and is used mainly in veterinary medicine. In human medicine, it is occasionally used to treat the symptoms of migraine. The elimination half-life of tolfenamic acid reported in different species is: dogs = 5.3 hours, cats = 10.8 hours, horses = 7.3 hours, cattle = 11.3 hours and man = 2.2 hours. Tolfenamic acid has long duration of action in different species, which require single daily

dosing. It is available in several formulations for oral and parenteral administration in animals.

Dose

Dogs & cats : 4 mg/kg, PO, once daily for 3 days given with food; may be repeated after 4 days.
: 4 mg/kg, SC or IM; may be repeated once after 24 hours, if required.
Cattle : 2 mg/kg, IM, once daily.
Pigs : 2 mg/kg, IM, as a single dose.

Mefenamic acid

Mefenamic acid is another anthranilic acid derivative that possesses analgesic, antipyretic and anti-inflammatory activities. Like meclofenamic acid, it inhibits PGs synthesis and antagonises certain actions of PGs as well. It has lower efficacy and may be useful in the relief of pain in conditions which do not ordinarily require strong analgesic. In humans, it is commonly used to treat pain, including menstrual pain.

Other anthranilic acid derivatives

Flufenamic acid, niflumic acid and etofenamic acid are other fenamates used in therapeutics. Veterinary proprietary preparations of niflumic acid are available for use in animals.

3. Sulphonanilide Derivatives

Nimesulide

Nimesulide is a sulphonanilide compound that possesses anti-inflammatory, analgesic and antipyretic activities. It is a relatively weak inhibitor of COX-1, but has significant inhibitory effect on the COX-2. It appears to exert its effects by other mechanisms also like inhibition of histamine release, reduced generation of superoxide by neutrophils, inhibition of platelet activating factor and free radical scavenging. There is evidence that nimesulide inhibits also release of tumour necrosis factor-alpha (TNF-α) and, thus, reduces the formation of cytokines. It also blocks the metalloproteinase activity of articular chondrocytes. The analgesic, antipyretic and anti-inflammatory activity of nimesulide has been rated comparable to other NSAIDs.

Nimesulide is well absorbed after oral administration and is highly bound (>95%) to plasma proteins. In humans, it has a relatively rapid onset of action, with significant reductions in pain and inflammation observed within 15 minutes from drug intake. Nimesulide is metabolised in the liver primarily to 4-hydroxynimesulide, which appears to be biologically active. The average half-life of nimesulide is humans is about 3 hours. Data on pharmacokinetics in animals are scarce.

Side effects of nimesulide are reported to be less frequent and involve the GI tract and the skin. Vomiting, diarrhoea, inappetance, haematochezia, lethargy and discoloration of serum and urine are some important side/adverse effects observed in dogs. Like most drugs in NSAID category, nimesulide is known to be hepatotoxic in rare but unpredictable cases and should be taken with care. Use of nimesulide is contraindicated in animals hypersensitive to the drug, in blood dyscrasias and pregnant or lactating bitches. Its use is not recommended in cats and in puppies less than 4 months of age or less than 3.5 kg body-weight. Nimesulide has been used primarily in human medicine and also veterinary medicine for short lasting painful inflammatory conditions. It appears to be particularly useful for patients those have allergic hypersensitivity to aspirin and other NSAIDs. Due to concerns about the risk of hepatotoxicity, particularly in children, nimesulide has been withdrawn from market in some countries.

Dose

Dogs : 5 mg/kg, PO, once daily for 3-5 days.

4. Alkalones

Nabumetone

Nabumetone is a newly developed pro-drug that possesses substantial anti-inflammatory, antipyretic and analgesic activities. Nabumetone itself is a weak inhibitor of cyclooxygenase enzyme, but after absorption it is converted in the liver to its main active metabolite 6-methoxy-2-naphthylacetic acid (6-MNA) which is a potent inhibitor of cyclooxygenase, in particular COX-2. In experimental animals, nabumetone appears to cause less gastric erosions, ulcers and bleeding than do other NSAIDs. Nabumetone, like other NSAIDs, is associated with several suspected or probable interactions which affect the action of other drugs. Nabumetone is used mainly in human medicine for the treatment of inflammation and pain those results from rheumatoid arthritis and osteoarthritis. Data on efficacy, pharmacokinetics and adverse effect profile in domestic animals are lacking.

III. AGENTS WITH ANALGESIC AND POOR ANTI-INFLAMMATORY ACTIVITY

1. Para-Aminophenol Derivatives/Aniline Derivatives

Paracetamol

Paracetamol (Acetaminophen) is a para-aminophenol derivative that possesses analgesic and antipyretic effects similar to aspirin, but is not an anti-inflammatory agent. Paracetamol is very popular for use in human patients because it is well tolerated and lacks many of the side effects of aspirin, but has limited application in veterinary practice mainly due to toxicity associated with paracetamol administration, especially in cats.

Mechanism of action

The main mechanism of action of paracetamol is considered to be the inhibition of cyclooxygenase (COX) enzyme, and recent findings suggest that it more selective for COX-2 than COX-1. Paracetamol mediates its analgesic-antipyretic effect mainly through central component by inhibition of cyclooxygenase enzyme in brain. It is a poor inhibitor of cyclooxygenase enzyme and PGs in the peripheral tissues. While the analgesic and antipyretic properties of paracetamol are comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is its failure to inhibit cyclooxygenase enzyme in the presence of high concentrations of peroxides which are found in inflammatory lesions. Further, it does not inhibit neutrophil activation, as do many other NSAIDs. Because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes. Paracetamol is also reported to modulate the endogenous cannabinoid system, which is involved in a variety of physiological processes including pain-sensation.

Pharmacological effects

Paracetamol has good analgesic and antipyretic effects, but has only weak peripheral anti-inflammatory activity. An anti-inflammatory effect can be demonstrated in animal models, but only at doses considerably in excess of those required for

analgesia. Paracetamol does not produce gastric irritation, erosion or bleeding that may occur after administration of salicylates. In humans, it has no effects on platelets, bleeding time or excretion of uric acid.

Pharmacokinetics

Pharmacokinetic data on paracetamol in domestic animals are limited. In humans, paracetamol is well absorbed orally and uniformly distributed in the body. It is bound to about 35% to plasma proteins in blood. Paracetamol is metabolised via a variety of pathways including conjugation with glucuronic acid (~60%), sulphate (~35%) and cysteine (3%). A small portion of paracetamol undergoes cytochrome P-450 mediated N-hydroxylation to form N-acetyl-p-benzoquinone imine, a highly reactive electrophilic intermediate metabolite. This metabolite normally reacts with nucleophilic sulphydryl group in glutathione and is excreted as mercapturic acid in urine (Figure 30.7). However, after ingestion of large doses of paracetamol, the metabolite is formed in amounts sufficient to deplete hepatic glutathione. In the presence of glutathione depletion, this metabolite covalently reacts with sulphydryl groups in hepatic and renal proteins and other macromolecules thereby resulting in hepatic inactivation of essential enzyme systems and cell death (i.e. hepatic and renal necrosis). Covalent binding to DNA is responsible for carcinogenicity and tumour formation. Cats have a reduced capacity for glucuronide conjugation and the paracetamol is converted easily to reactive electrophilic metabolite in this species. Dogs also do not metabolise paracetamol as efficiently as humans. Dogs apparently exhibit dose-dependent metabolism (saturable).

Side effects/Adverse effects

Paracetamol is safe in recommended therapeutic doses and is well tolerated in humans, but its use in dogs may cause renal, hepatic, GI and haematological effects. Paracetamol in high doses may cause severe liver and kidney damage through electrophilic metabolite. Paracetamol poisoning is common in cats because it is frequently administered by owner themselves due to easy availability at homes. Toxic manifestation of paracetamol includes cyanosis, Heinz body anaemia, methaemoglobinaemia, jaundice, liver failure and facial oedema. Paracetamol acting via active metabolites oxidises haemoglobin to methaemoglobin and sulphaemoglobin, shortens life of erythrocytes, and can cause frank haemolysis. The cyanotic conditions occur due to hypoxia associated with conversion of haemoglobin to methaemoglobin. Haemolysis of red blood cells is responsible for anaemia (Heinz bodies) and haemoglobinuria. Jaundice is attributed to both lysis of erythrocytes and hepatic necrosis. High doses of paracetamol are often fatal in cats.

Treatment: Treatment of toxicity or overdosage of paracetamol in cats and dogs involve standard detoxification and supportive measures. Acetylcysteine (150 mg/kg, IV or PO initially followed by 70 mg/kg, PO every 4 hours for 5 to 7 treatments) can reduce extent of liver injury or methaemoglobin in dogs and cats by providing an alternate substrate for conjugation with the reactive metabolite of paracetamol.

Contraindications and precautions

Paracetamol is contraindicated in cats at any dosage. In dogs, it is not recommended to use in hepatic or renal toxicity, or haematological abnormalities including anaemia. It should be avoided during the intermediate post-operative phase (first 24 hours) in dogs due to an increased risk of hepatotoxicity developing.

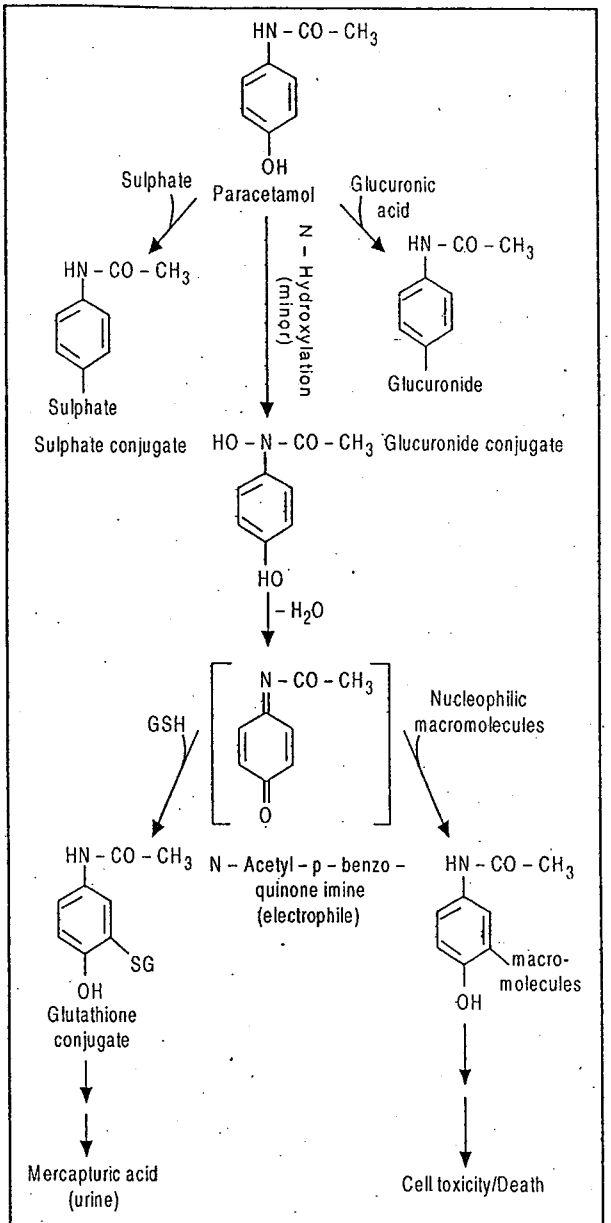


Fig. 30.7. Biotransformation of paracetamol (see text for detail).
Abbreviations : GSH = glutathione.

Drug interactions

Paracetamol should not be used for post-operative analgesia in animals those have received halothane or other hepatotoxic anaesthetics. Large doses may potentiate effects of oral anticoagulants. Analgesic action of aspirin and paracetamol is reported to be additive.

Clinical uses

Paracetamol is not frequently used in veterinary practice. Occasionally, it can be used as an oral analgesic in dogs. In conditions of more severe pain, it may be used in combination with oral codeine phosphate. In humans, it is one of the best drugs to be used as antipyretic and most commonly used 'over the counter' analgesic, where anti-inflammatory action is not required.

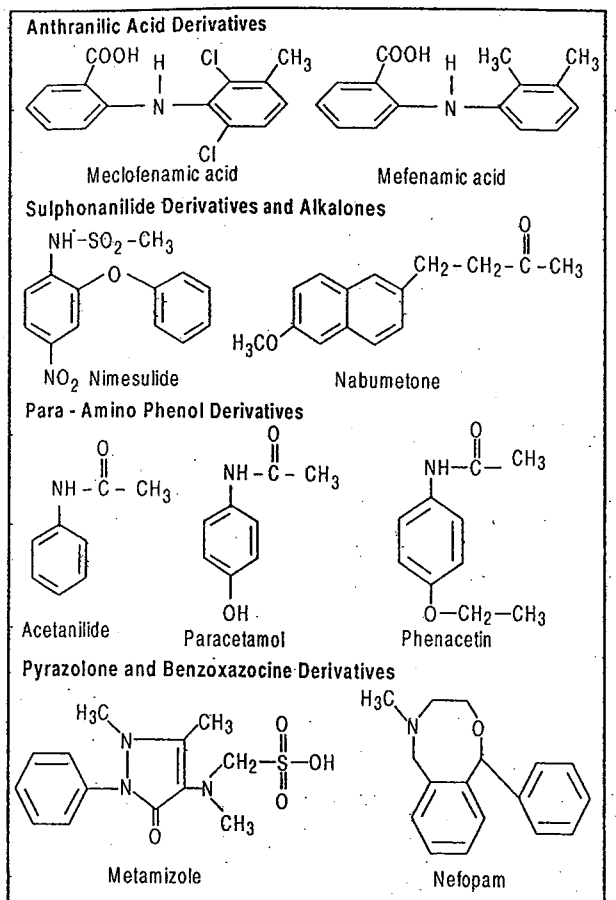


Fig. 30.8. Chemical structures of selected anthranilic acid, sulphonanilide, para-amino phenol, pyrazolone and benzoxazocine derivative non-steroidal anti-inplammatory drugs.

Dose

Dogs : 10 – 15 mg/kg, PO, 2 times daily.

Acetanilide and Phenacetin

Acetanilide and phenacetin (acetophenetidin) are aniline derivatives and chemically related to paracetamol. Acetanilide and phenacetin form paracetamol as one of their metabolites after biotransformation, which may account for their major pharmacological actions. Acetanilide in addition forms aniline as a metabolite, which is highly toxic and readily converts haemoglobin into methaemoglobin. Phenacetin, once incorporated in the well-known APC (aspirin, phenacetin and caffeine) tablet, has been associated with high incidence of necrosis of renal papilla in man and is, therefore, not freely available. Both acetanilide and phenacetin are now banned in many countries, including India, and are not used in therapeutics.

2. Pyrazolone Derivatives

Metamizole

Metamizole, commonly known by the brand names of analgin and novalgin, is a pyrazoline derivative that is frequently used as analgesic and antipyretic agent in both human and veterinary medicines. It acts mainly by blocking synthesis of prostaglandins in the CNS. The decrease of the prostaglandins production in the periphery and respective decrease of nerve endings sensitivity plays a relatively smaller role. In contrast to the other NSAIDs, metamizole is reported to stimulate the release of β-endorphins, explaining its activity in cases of visceral pain. It also has a slight spasmolytic activity on the smooth muscle cells of the biliary and urinary tracts and also on the muscle of the uterus. In contrast to phenylbutazone and other pyrazolones, metamizole is a poor anti-inflammatory and not uricosuric. Two of its metabolites viz. 4-aminoantipyrin and 4-methylaminoantipyrin are active and possess significant analgesic and antipyretic activities.

Long-term use of metamizole should be avoided as it may result in agranulocytosis and neutropenia. It also has a tendency to increase bleeding as a result of suppression of formation of prothrombin. Overdosage may result in convulsive seizures. Use of metamizole is contraindicated in cases of hypersensitivity to metamizole and other pyrazolone derivatives, acute hepatic porphyria, severe renal or hepatic diseases, blood diseases (e.g. aplastic anaemia, leucopenia and agranulocytosis) and pregnancy. Metamizole potentiates the effects of other NSAIDs and drugs possessing CNS depressant activity. Concurrent administration with chlorpromazine is related with a risk of severe hypothermia. In veterinary medicine, it has been used to treat equine colic and other conditions of GI spasms or hypermotility in both small and large animals. Metamizole may also be used for the treatment of fever and pains of different origin and variable intensity such as arthralgia, neuralgia, myositis and mild to moderate visceral pain. Metamizole solution is irritant, so it is preferably given by IV route. Several countries have banned metamizole due to the possible risk of a rare but fatal agranulocytosis in humans, while others have maintained its free availability over the counter.

Dose

Horses : 20 – 50 mg/kg, IV or IM, 3 times daily.

Pigs : 15 – 50 mg/kg, IV or IM, 3 times daily.

Cattle : 20 – 40 mg/kg, IV or IM, 3 times daily.

Propyphenazone

Propyphenazone is another pyrazolone derivative that is similar in properties to metamizole. Unlike metamizole, agranulocytosis has not been reported. Propyphenazone is claimed to be better tolerated than metamizole.

3. Benzoxazocine Derivatives

Nefopam

Nefopam is a centrally-acting non-opioid analgesic drug of the benzoxazocine chemical class. The mechanism of action of nefopam is not well understood, although blockade of prostaglandins synthesis does appear to be the primary mechanism. Inhibition of serotonin, dopamine and norepinephrine reuptake is thought to be involved in its analgesic effects. Other modes of action such as modulation of histamine H_3 and glutamate receptors, and blockade of voltage-gated sodium channel may also be involved in some of its action. Nefopam acts rapidly with an efficacy approaching morphine, yet it has no opioid actions. It is widely used in human medicine, mainly in European countries, for the relief of moderate to severe pain as an alternative to opioid analgesic drugs. Favourable results have been obtained in short lasting musculo-skeletal pain not responding to other NSAIDs.

SELECTIVE COX-2 INHIBITORS

Selective COX-2 inhibitors (COX-2Is or Coxibs) are a novel class of non-steroidal anti-inflammatory drugs which directly target cyclooxygenase-2, the isoenzyme mainly responsible for inflammation and pain. Selective inhibition of COX-2 improves gastro-intestinal tolerance and minimizes some side/adverse effects

observed with most non-selective COX-inhibitors. However, COX-2 selectivity does not seem to reduce other adverse effects of NSAIDs (e.g. renotoxicity). Over the last 10 years the clinical use of COX-2 inhibitors has exponentially grown and some drugs including firocoxib, deracoxib, mavacoxib and robenacoxib have been introduced for veterinary use.

1. Furanone Derivatives

Rofecoxib

Rofecoxib is a diaryl-substituted furanone non-steroidal anti-inflammatory drug. It exhibits anti-inflammatory, antipyretic and analgesic activities, which have been attributed to selective inhibition of COX-2. At therapeutic doses, rofecoxib does not inhibit COX-1 and does not alter platelet functions. Aside from the reduced incidence of gastric ulceration, rofecoxib exhibits a similar adverse effect profile to other NSAIDs.

Rofecoxib was introduced in market in 1999 for the treatment of osteoarthritis, acute pain and dysmenorrhoea in human patients. It gained widespread acceptance among physicians treating patients with arthritis and other conditions causing chronic or acute pain. However, it was withdrawn from the market by the manufacturer in 2004 because of concerns about increased risk of heart attack and stroke associated with long-term, high-dosage use.

Firocoxib

Firocoxib is a selective COX-2 inhibitor non-steroidal anti-inflammatory drug approved for use in veterinary medicine. Like other NSAIDs, firocoxib produces analgesic and anti-inflammatory effects by inhibiting synthesis of prostaglandins. Firocoxib *in vitro* assays has demonstrated a more COX-1 sparing action compared to the classical NSAIDs and is believed to be a selective inhibitor of COX-2. After oral administration firocoxib is absorbed to the extent of 40% in dogs, 55-70% in cats and 80% in horses. Presence of food delays but does not diminishes the extent of absorption. Like other NSAIDs, it is highly (96-98%) bound to plasma proteins. Firocoxib has a half-life of about 8 hours in dogs, 9-12 hours in cats and 30-40 hours in horses.

Firocoxib appears to be well tolerated in animals with minimal side effects on the GI tract. Vomiting, diarrhoea, nausea, ulcers and GI erosions may occur at higher or frequent doses. Behaviour changes have been reported in dogs and horses, but they are rare. Periportal fatty hepatic changes have been reported in some young animals treated with firocoxib. The contraindications, precautions and drug interactions of firocoxib are similar to those listed for other NSAIDs. Firocoxib has been recommended for treatment of acute and chronic pain and inflammation in dogs. In horses, it is mainly used for osteoarthritis. In cats, firocoxib has demonstrated good antipyretic effect in some febrile conditions. However, its treatment in cats is limited to short-term use or long-term use at low doses.

Dose

Dogs : 5 mg/kg, PO, once daily.
Cats : 1.5 mg/kg, PO, once daily.
Horses : 0.1 mg/kg, PO, once daily for up to 14 days.

2. Pyrazole Derivatives

Celecoxib

Celecoxib is a diaryl-substituted pyrazole NSAID that selectively inhibits COX-2. However, the COX-2 selectivity of celecoxib is modest (10-20 fold). Similar to rofecoxib, it exerts, anti-inflammatory, analgesic and antipyretic effects, with minimal gastrointestinal adverse drug reactions (e.g. stomach ulcers). In humans, celecoxib is slowly absorbed, 97% plasma protein bound, metabolized in liver and has half-life of about 10 hours. Celecoxib is primarily used in human medicine in the treatment of osteoarthritis, rheumatoid arthritis, acute pain and menstrual symptoms.

Deracoxib

Deracoxib is a non-steroidal anti-inflammatory drug of the coxib class, approved for use in veterinary practice only. Similar to celecoxib, it produces analgesic, antipyretic and anti-inflammatory effects by selectively inhibiting COX-2 form of enzyme. High doses can also cause COX-1 inhibition, which in turn can cause enhance side effects associated with deracoxib. Deracoxib has oral absorption of about 90% in dogs. It is highly plasma protein bound with a half-life is about of 3 hours. Food can delay, but does not diminish overall absorption of the drug.

Gastrointestinal upsets such as nausea, vomiting, diarrhoea and inappetance are important side/adverse effects associated with deracoxib, particularly during long-term use or in sensitive animals. Other side effects may include depression, lethargy, increase in drinking or urination, jaundice, vomiting, bloody or black stools, pale gums, hot spots, increased respiration (fast or heavy breathing), incoordination and behaviour changes. Gastric and duodenal ulcers have also been reported from use of deracoxib in dogs. Such side effects are more likely to occur if this drug is used in combination with other NSAIDs or with steroids; therefore deracoxib should not be administered with other NSAIDs or steroids. Use of deracoxib should be avoided or administered with extreme caution in dogs which are hypersensitive to deracoxib or other NSAIDs and in animals with gastro-intestinal ulcers, renal disease, hepatic disorders, hypoproteinaemia, dehydration or cardiac disease. Safety has not been established in pregnant or nursing bitches, so deracoxib should not be administered to such dogs.

Deracoxib is primarily indicated to treat osteoarthritis in dogs. It may also used to decrease pain, inflammation and fever associated with other conditions such after surgery. It is not approved or recommended for use of pain control in cats. Use of deracoxib in large animals is not reported. Deracoxib is available in 25 and 100 mg chewable tablets, which have added beefy flavour to increase palatability.

Dose

For osteoarthritis

Dogs : 1 – 2 mg/kg, PO, once daily.

For post-operative pain

Dogs : 3 – 4 mg/kg, PO, once daily for 7 days.

Mavacoxib

Mavacoxib is a non-steroidal anti-inflammatory drug (NSAID) of the coxib class. It acts by selective inhibition of COX-2-mediated prostaglandin synthesis. At higher doses, mavacoxib also inhibits COX-1 and may become non-selective in action. It possesses analgesic and anti-inflammatory properties. Mavacoxib is well absorbed after oral administration with bioavailability of 87% in fed dogs and 46% in fasted conditions. After absorption, it binds to plasma proteins up to 98%, extensively distributes in

body and excretes mainly via bile. The rate of body clearance of mavacoxib is very slow in dogs with mean elimination half-life of 39 days. In some cases the elimination half-life may extend up to 80 days. The reason for this long half-life is unknown.

Mavacoxib may produce side/adverse effects such as such as loss of appetite, diarrhoea, vomiting and apathy. Impaired renal functions have occasionally been reported, which in rare cases may be fatal. The safety of mavacoxib has not been established during pregnancy and lactation; therefore, the drug should not be used in pregnant, breeding or lactating animals. Due to its prolonged half-life, mavacoxib should not be administered to animals which might not tolerate prolonged NSAID exposure. Similarly it should not be used in dogs less than 12 months of age and/or less than 5 kg body weight. No drug interaction studies have been reported for mavacoxib, but similar to other NSAIDs it should not be administered simultaneously with other NSAIDs or glucocorticoids.

Mavacoxib has been used for the treatment of pain and inflammation associated with degenerative joint disease in dogs in cases where continuous treatment exceeding one month is indicated. A maximum administration of 6.5 months continuous therapy is recommended so as to manage plasma levels of mavacoxib in animals which exhibit reduced elimination. Mavacoxib is commercially available in 6, 20, 30, 75 and 95 mg chewable tablets for dogs.

Dose

Dogs : 2 mg/kg, PO, given immediately before or with meal. The treatment should be repeated 14 days later, thereafter the dosing interval is one month. The treatment cycle should not exceed 7 consecutive doses (6.5 months).

3. Isoxazole Derivatives

Valdecoxib

Valdecoxib is a diaryl-substituted isoxazoles NSAID with selective inhibitory action on COX-2 enzyme. Valdecoxib has a very strong (about 90 fold) affinity for COX-2 over the COX-1 enzyme. It is mainly recommended in human practice for the treatment of osteoarthritis, rheumatoid arthritis, and painful menstruation and menstrual symptoms. Presently it has been removed from the market due to concerns about possible increased risk of heart attack and stroke.

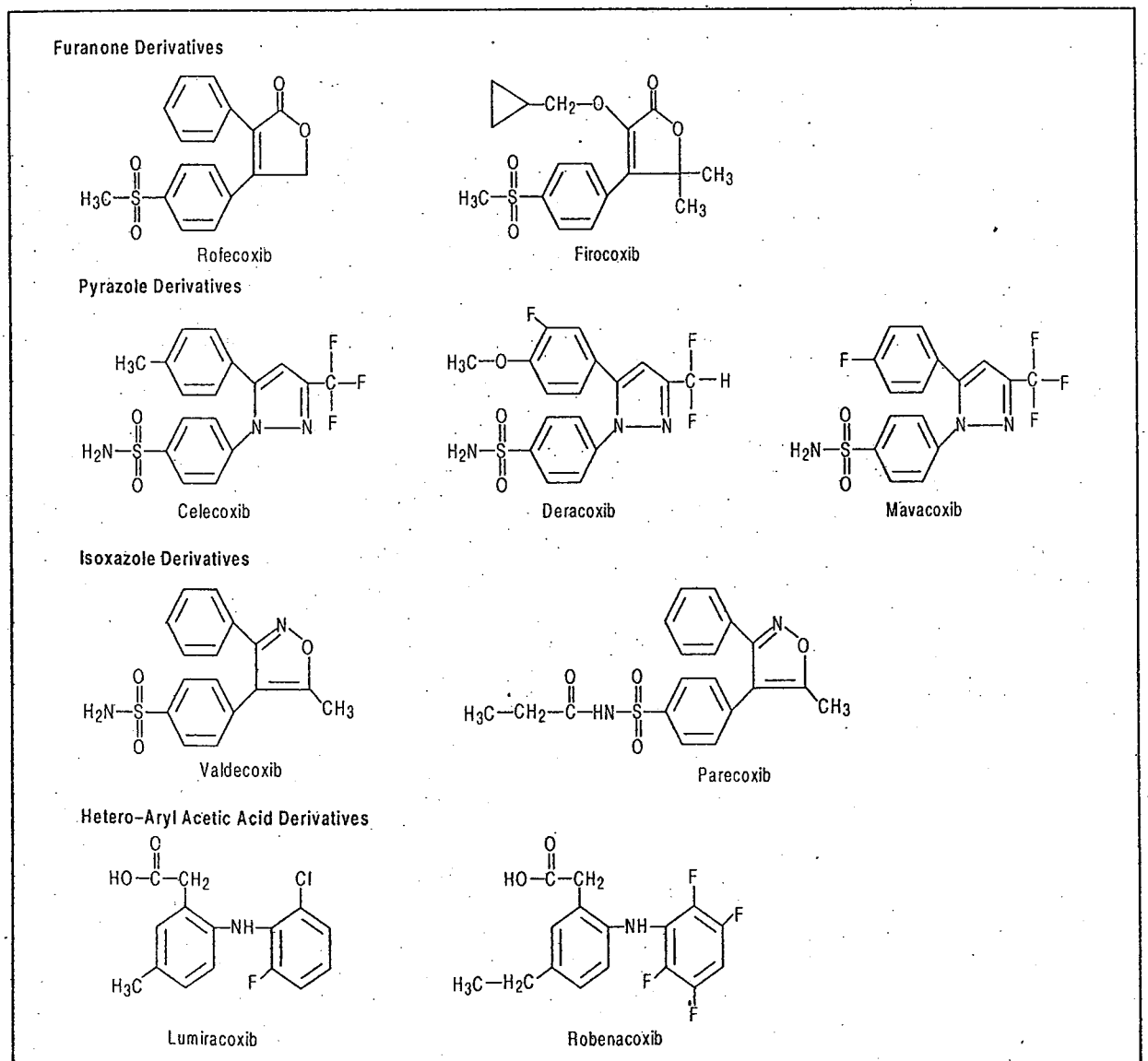


Fig. 30.9. Chemical structures of selected COX-2 inhibitor non-steroidal anti-inflammatory drugs.

Parecoxib

Parecoxib is a water soluble and injectable prodrug of valdecoxib. As it is injectable, it can be used perioperatively when patients are unable to take oral medications. Its analgesic and anti-inflammatory efficacy is comparable to ketorolac. However, unlike ketorolac, parecoxib has no effect on platelet function and therefore does not promote bleeding during or after surgery. Parecoxib is used in primarily human medicine for relief of post-operative or similar short-term pain.

4. Hetero-Aryl Acetic Acid Derivatives

Lumiracoxib

Lumiracoxib is a COX-2 selective inhibitor non-steroidal anti-inflammatory drug. Lumiracoxib is an analogue of diclofenac, making it a member of aryl acetic acid class of NSAIDs. It has several distinctive features such as its structure is different from other commonly used COX-2 inhibitors like celecoxib; it binds to a different site on the COX-2 receptor than do other COX-2 inhibitors; it is the only acidic coxib; and it has the highest COX-2 selectivity of any NSAID. Presently, lumiracoxib has been withdrawn from the market in several countries, mostly due to hepatotoxicity concerns.

Robenacoxib

Robenacoxib is a COX-2 selective non-steroidal anti-inflammatory drug belonging to the aryl acetic acid class. It inhibits the pain-causing COX-2 enzyme while sparing the protective actions of COX-1 enzymes. Robenacoxib appears to be tissue selective, with significant action at the inflamed and painful sites. Gastric upsets (e.g. nausea and vomiting) are common side/adverse effects of robenacoxib. Robenacoxib is contraindicated in animals suffering from GI ulcerations, renal inefficiency and hepatic disorders. Its use is not recommended in animals which are dehydrated, hypovolaemic or hypotensive. If use cannot be avoided, such animals require careful monitoring and fluid therapy. Robenacoxib is indicated in small animals for pain and inflammation due to musculo-skeletal disorders. It may also be used for other painful conditions such as orthopaedic or soft tissue surgery. It is available in both oral tablet and injectable solutions for use in dogs and cats.

Dose

Dogs	:	1 – 2 mg/kg, PO, once daily.
Cats	:	1 mg/kg, PO once daily.
Dogs & cats	:	2 mg/10 kg, SC, approximately 20 minutes before start of surgery.

5. Miscellaneous Drugs

Etoricoxib

Etoricoxib is a bipyridine NSAID approved for use in human medicine. It is approx. 106 times more selective for COX-2 inhibition over COX-1. Its therapeutic indications include rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout. The half-life of etoricoxib in human beings is about 24 hours.

III. MIXED COX AND 5-LOX INHIBITORS

Tepoxalin

Tepoxalin belongs to a new class of NSAIDs causing dual inhibition of both COX-1/COX-2 and 5-LOX enzymes. It is approved for veterinary use, primarily in dogs, as an anti-inflammatory and analgesic.

Mechanism of action

Tepoxalin is basically a pro-drug that is converted *in vivo* into an acid metabolite, which inhibits the cyclooxygenase enzyme. It appears to be more COX-1 selective than COX-2 selective. Tepoxalin produces most of its action through this metabolite. Unlike activity on the COX enzyme, the inhibitory action of tepoxalin on 5-LOX is through the parent compound and not the active metabolite. Inhibitions of both COX and 5-LOX enzymes decrease synthesis of prostaglandins and leukotrienes.

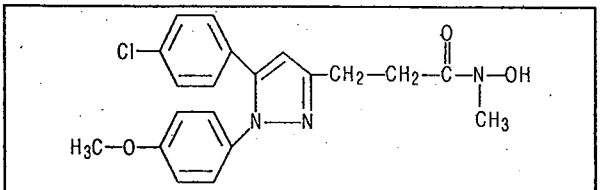


Fig. 30.10. Chemical structure of tepoxalin.

Pharmacological effects

The pharmacological effects of tepoxalin are similar to other commonly used NSAIDs. However, dual inhibition of COX and 5-LOX provides a broader spectrum of anti-inflammatory activity than that of pure COX inhibitors. Inhibition of 5-LOX may reduce the gastrointestinal toxicity associated with other non-steroidal anti-inflammatory drugs, which only inhibit COX (cyclooxygenase).

Pharmacokinetics

Tepoxalin is administered by oral route and its absorption is more in presence of food than in the empty stomach. It is metabolized in body to an acid metabolite that causes most of the pharmacodynamic effects. Both tepoxalin and it active metabolite are highly bound to plasma proteins. Both parent compound and active metabolite are primarily excreted in faeces. In dogs, tepoxalin has half-life of 2 hours and its active metabolite a half-life of 13 hours.

Side/Adverse effects

Important side/adverse effects associated with tepoxalin use in dogs include nausea, vomiting, inappetance, diarrhoea, lethargy, haematochezia, gastric ulcers and erosion of GI tract. Occasionally alopecia and erythema have also been observed in dogs. Renal toxicity, especially in dehydrated animals or animals with pre-existing renal disease, has been shown in some cases.

Contraindications and precautions

The contraindications and precautions of tepoxalin use in dogs are in general similar to those of other NSAIDs. Animals with pre-existing GI problems or renal disease may be at greater risk of side/adverse effects of tepoxalin.

Drug interactions

Drug interactions have not been fully studied for tepoxalin, but it may follow other NSAIDs. Its concurrent administration along with diuretics, anticoagulants or plasma-protein bound drugs should be avoided. Operator should ensure hands are dry before handling the product.

Clinical use

Tepoxalin has been developed for use in veterinary medicine, mainly in dogs. It is primarily used to reduce inflammation and relief of pain caused by musculoskeletal disorders such as

osteoarthritis. It is also used for pain associated with surgery. Because of dual action of tepoxalin, it has been investigated for treating other inflammatory conditions in dogs and cats. It is available as a rapidly dissolving oral tablet and should be administered with food.

Dose

Dogs : 10-20 mg/kg, PO, once daily administered within 1-2 hours of feeding or along with feed.

Lecofelone

Licofelone is a dual COX/LOX inhibitor with effects similar to those of tepoxalin. Licofelone is both an analgesic and an anti-inflammatory. It is primarily recommended for human use for the treatment for osteoarthritis.

OTHER ANTI-INFLAMMATORY AND CHONDROPROTECTIVE DRUGS

In addition to the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) discussed above, many other classes of drugs are commonly used in therapeutics to suppress or abolish pain and inflammation in soft tissues and joints. One such class of pharmaceuticals is chondroprotective drugs which are used to retard degradation of cartilage and promote chondrocyte synthesis of collagen and proteoglycans in the joints. These anti-inflammatory, chondroprotective and/or related drugs are often useful in arthritis, particularly osteoarthritis, and some other inflammatory conditions associated with various diseases in animals.

1. Dimethyl sulphoxide

Dimethyl sulphoxide (DMSO) is an organosulphur compound with the formula $(CH_3)_2SO$. It is commonly used in veterinary medicine as a topical (mainly) and parenteral anti-inflammatory drug. It is also extensively used as an extractant in biochemistry and cell biology.

Properties

Dimethyl sulphoxide is a clear to yellow liquid that was developed as an industrial solvent. It is extremely hygroscopic and is miscible with water, alcohol and many organic solvents. It readily dissolves both water soluble and lipid soluble drugs and is occasionally used to transport drugs. The industrial grade of the chemical may contain potentially toxic impurities and should not be used for medical purposes. Dimethyl sulphoxide must be stored in airtight glass containers and away from light, because it may react with some plastics in the presence of air. A 2.15% solution is isotonic with serum.

Mechanism of action

The exact mechanism of action of DMSO as an anti-inflammatory drug is unknown. It is suggested that DMSO traps free radical hydroxide and its metabolite dimethyl sulphide (DMS) traps free radical oxygen. Dimethyl sulphoxide also increases membrane stabilisation produced by corticosteroids, reduces pain impulse conduction, movement of phagocytic cells into an inflamed area, increases blood flow by local vasodilatation, protects endothelium and reduces thrombus formation. These actions appear to explain some of the anti-inflammatory, analgesic, chondroprotective and anti-ischemic activities of DMSO.

Pharmacological effects

Dimethyl sulphoxide possesses anti-inflammatory, analgesic and some antifungal and antimicrobial properties. It appears to be more effective an anti-inflammatory agent when used for acute inflammation than chronic inflammation. Its analgesic effects have been compared to those produced by opioid analgesics and is efficacious for both acute and chronic musculo-skeletal pain. DMSO slows and/or reduces platelet aggregation but its effects on blood coagulation are not clear. Topical application causes local vasodilatory effect, which probably occurs due to histamine release from mast cells. Its topical application can cause some irritation with increase in skin temperature. Dimethyl sulphoxide also apparently has some anticholinesterase activity and blocks the synthesis of prostaglandins. It induces diuretic effect independent of the route of administration.

Pharmacokinetics

Dimethyl sulphoxide is well absorbed within 5 minutes of topical application as it easily penetrates most membranes and intact skin. Once absorbed, it distributes widely in most tissues within 20 minutes and can be detected in systemic secretions, excretions and internal compartments. It is metabolised to dimethyl sulphide (DMS) in small animals and dimethyl sulphide and dimethyl sulphone in bovines, which are excreted mainly in the urine; some biliary and respiratory excretion also takes place. In horses, the serum half-life is reported to be approximately 9 hours. It has an unusual property of being secreted onto the surface of the tongue after contact with the skin and causing a garlic-like taste in the mouth.

Side effects/Adverse effects

Dimethyl sulphoxide is considered to be a safe drug when used in recommended dosage for short-term. Transient local effects like cutaneous dehydration, erythema, oedema and pruritus due to histamine release, and garlic or oyster-like breath odour are the most likely adverse effects. These effects resolve quickly when therapy is discontinued. Extended use of DMSO at high doses has been correlated with induction of cataract of the lens and myopia in dogs. These effects are slowly reversible after the drug is discontinued. Haemolysis and haemoglobinuria occurs in horses when DMSO is administered IV, particularly at concentrations of 20% or more. Mild cholinesterase inhibition, immuno-suppression and diuresis are other adverse effects. Incidence of hepatotoxicity and renal toxicity has also been reported for various species and dosages. Dimethyl sulphoxide is a potential teratogen in the first 3 weeks of pregnancy. Very high doses may produce toxicity that is manifested by prostration, transient convulsions, opisthotonus, dyspnoea and coma.

Contraindications and precautions

Dimethyl sulphoxide should be used with extreme caution in animals suffering from dehydration or shock as its diuretic and peripheral vasodilatory effects may exacerbate these conditions. Operators should wear impervious gloves because DMSO easily penetrates intact skin and produces a garlic-like taste in the mouth. It should only be applied to clean and dry skin that is free of other medications. Contact with eye should be avoided. It should be used in well-ventilated area to lessen chances of inhalation. Its administration to pregnant animals should be avoided. It should not be mixed with any other substance without proper knowledge.

Drug interactions

Use of organophosphates and other anticholinesterase agents with DMSO should be avoided because it potentiates the anticholinesterase activity. Toxicity of DMSO is greatly enhanced by general anaesthetics. Dimethyl sulphoxide may prolong the effects of alcohol because it inhibits alcohol dehydrogenase enzyme.

Clinical uses

Dimethyl sulphoxide is primarily approved for topical use in the treatment of acute swelling due to trauma in horses and in an otic preparation for the relief of pain and inflammation due to acute or chronic otitis in dogs. Other proposed uses of DMSO include the treatment of ophthalmic conditions, dermatitis, acute and chronic musculo-skeletal conditions, tissue trauma, burns, post-surgical oedema, reduction of granulation tissue and the improvement of healing in ulcerative processes of the skin. It has also been used in the acute treatment of brain and spinal cord trauma, where it is said to scavenge oxygen radicals, decrease thrombus formation and improve tissue perfusion.

Dimethyl sulphoxide is an important and potential carrier of therapeutic agents across the skin and into the systemic circulation, but it requires careful consideration. It is frequently compounded with antifungal medications, enabling them to penetrate not just skin but also toe and nails. As it inhibits the alcohol dehydrogenase enzyme, it may be considered useful in the treatment of ethylene glycol poisoning where it decreases conversion of ethylene glycol into toxic metabolites. Dimethyl sulphoxide should be topically applied with clean and sterile cotton to minimise the chances for contamination.

Dose

For local inflammation (DMSO 70-90% solution)

Dogs & cats : Topical application over the affected area 3 to 4 times daily for not more than 2 weeks. Total daily dose should not exceed 20 ml (or 20 g).

Horses : Topical application over the affected area 2 to 3 times daily for not more than 30 days. Total daily dose should not exceed 100 ml (or 100 g).

For systemic effects (cerebral oedema/spinal cord injury)

Dogs, cats & horses : 1 g/kg, as a 10-20% solution in 5 percent dextrose, slow IV over 30 minutes, once daily for 1 to 3 days.

2. Hyaluronate sodium/Hyaluronic acid

Hyaluronate sodium is the sodium salt of hyaluronic acid (HA) that is a naturally occurring high viscosity mucopolysaccharide. Because the molecule typically exists *in vivo* in its polyanionic form, it is also commonly referred to as hyaluronan. High concentrations of HA are found naturally in the synovial fluid, vitreous of eye and umbilical cord from where it is collected, extracted and purified for commercial use. Hyaluronic acid is available commercially as low molecular weight and high molecular weight preparations.

Chemistry and physiological effects

Hyaluronic acid (HA) is a linear polysaccharide. It is a polyanionic, non-sulphated glycosaminoglycan consisting of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine linked by (1, 3) glycosidic bonds. These units are then linked by (1, 4) glycosidic bonds to form long, unbranched chains consisting of between 10,000 and 20,000 disaccharide units. The average molecular weight of synovial fluid HA is between 2 - 6 x 10^6 Da in man and animals and is chemically identical regardless of the species. Hyaluronic acid is a major component of synovial fluid and it accounts for the high viscosity and thixotropic properties of the synovial fluid. In articular cartilage, HA is linked to proteoglycan molecules to form the aggregated proteoglycan matrix (protein-hyaluronate complex) that provides a thin protective layer on surface of cartilage. The net actions of HA in joints are a lubricating and cushioning effect and a reduction of protein and cellular influx into the joint.

Mechanism of action

The exact mechanism of action through which exogenous hyaluronic acid exerts its effect is not clear. It has been demonstrated that in some arthritides, especially osteoarthritis, joints have decreased viscosity of synovial fluid due to depolymerisation of HA which initiates a chain of events leading to or exacerbating degenerating changes. In the early stages of some arthritides, articular cartilage degradation occurs and there is loss of proteoglycans, hyaluronic acid and later collagen. Exogenous administration of HA increases viscosity of synovial fluid (called viscosupplementation) and retards cartilage degradation by enhancing binding of hyaluronate to cartilage proteoglycans. Hyaluronnic acid also reduces leucocyte migration, inhibits lymphocyte activation, suppresses phagocytic activity, reduces permeability of synovial membranes, and increases proteoglycan synthesis by chondrocytes. Hyaluronic acid is also reported to have direct anti-inflammatory effects in joints by scavenging free radicals and suppressing prostaglandins (mainly PGE_2) synthesis and cytokine interactions with cells. Increased synthesis of hyaluronic acid in synoviocytes by HA itself has been demonstrated.

Pharmacological effects

Intra-articular administration of HA decreases pain and improves joint mobility and performance in equines. In an equine model of osteoarthritis based on bilateral osteochondral fractures of the radial carpal bone, IV hyaluronate decreased synovial fluid protein, suppressed inflammatory synovium changes and reduced lameness. Although data on HA in dogs are inadequate, HA is reported to have a chondrostabilising effect on canine articular cartilage.

Pharmacokinetics

Pharmacokinetic data on hyaluronic acid in animals are not adequate. The half-life of exogenous HA administered intra-articularly into a normal equine joint is estimated at 96 hours. The plasma half-life of HA in a rabbit model following IV injection is between 2.5 to 4.5 minutes. Both the liver and articular tissues are capable of HA degradation.

Side effects/Adverse effects

Hyaluronate sodium does not produce any serious side effect. Some animals may develop local reactions manifested by heat, swelling and/or effusion after intra-articular injection, which generally subside within 24 to 48 hours. When used in combination with other drugs, incidence of flares may be high. These adverse effects can be minimised if HA is administered correctly. Systemic adverse effects have not been reported with HA either in recommended or with over doses.

Contraindications and precautions

There are no specific contraindications to HA use. It should not be administered intra-articularly through skin that has been recently blistered or has excessively scurf and counterirritants on it. No more than 2 joints should be treated at one time. Only a qualified person using aseptic techniques should administer HA.

Drug interactions

Specific drug interactions have not been noted with HA.

Clinical uses

Hyaluronic acid or Hyaluronan is useful in the treatment of various arthritides, articular disorders and navicular disease. It is indicated in equines for carpal and fetlock joint dysfunctions, but not when joint sepsis or fracture is suspected. It is particularly more useful for synovitis not associated with severe degenerative disease. High molecular weight preparations are recommended when synovial adhesions and pannus are to be avoided because they inhibit proliferation of synovial fibroblasts. Hyaluronic acid can be injected directly into an affected joint, or intravenously for less localized disorders. It may cause mild heating of the joint if directly injected, but this does not affect the clinical outcome.

Hyaluronic acid has many other uses in medical sciences. Some skin lotions containing sodium hyaluronate are used to treat dry and scaly skin (xerosis) such as that caused by atopic dermatitis (eczema). HA is gaining popularity as a biomaterial scaffold in tissue engineering research due to its high biocompatibility and its common presence in the extracellular matrix of tissues. HA levels are often used as a tumour marker for some cancers such as prostate and breast cancer and may also be used to monitor the progression of the disease as its levels correlate well with malignancy and poor prognosis. Hyaluronic acid may be used postoperatively to induce tissue healing, notably after cataract surgery. It is also incorporated in some anti-adhesive products such as Hyalobarrier, widely used in pelvic and abdominal surgery to prevent postoperative adhesions.

Dose

Horses	:	20 – 40 mg/joint, intra-articularly. Dose may be repeated at 40 mg (total dose), IV, if required.
Dogs	:	3 – 5 mg/joint (of high molecular preparation), intra-articularly. Dose may be repeated at weekly intervals, if required.

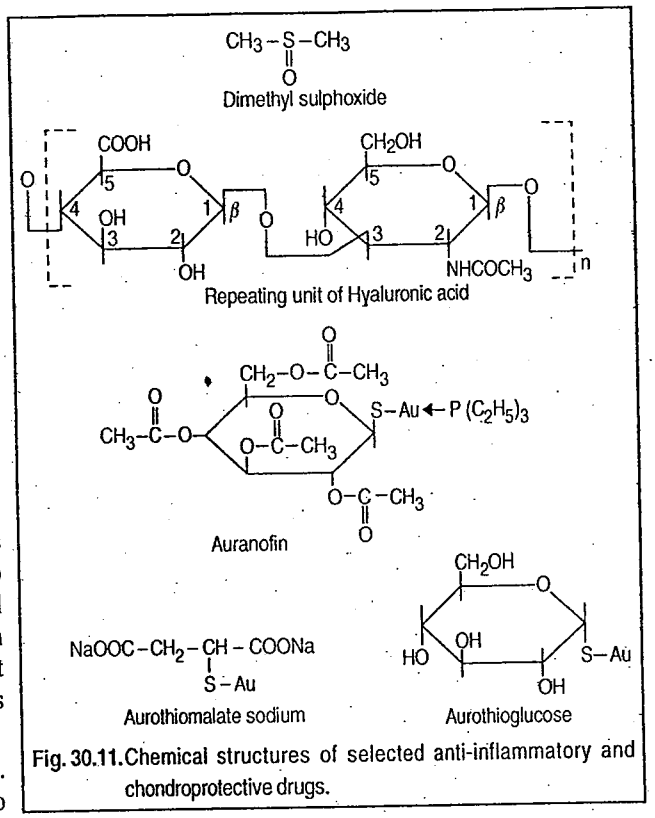


Fig. 30.11. Chemical structures of selected anti-inflammatory and chondroprotective drugs.

3. Glycosaminoglycans

Polysulphated glycosaminoglycan

Polysulphated glycosaminoglycan (PSGAG) is a compound that is chemically similar to heparin and sulphated glycosaminoglycan molecules which form part of the cartilage matrix. It is extracted from bovine trachea and lungs. PSGAG is a veterinary product approved for use in dogs and horses for the control of signs associated with non-infectious degenerative or traumatic arthritis.

Chemistry and physiological effects

Polysulphated glycosaminoglycan (Mol. Wt. 10,000) is a polymer compound mostly of chondroitin sulphate (CS). The chondroitin sulphates are glycosaminoglycans composed of alternating sulphated residue of uronic (β, D-glucuronic) acid and α-D-N-acetyl galactosamine linked by glycosidic β(1, 3) bonds. There is an average of 3.25 sulphate groups per repeating disaccharide units of CS. Glycosaminoglycans form the side chains of the core proteins of aggrecan (the large aggregating proteoglycan) and biglycan, decorin and fibromodulin (the small nonaggregating proteoglycan) of articular cartilage. Glycosaminoglycans also form the repeating units of hyaluronic acid (Figure 30.11).

Mechanism of action

Polysulphated glycosaminoglycan binds to damaged cartilage matrix presumably because of high negative charge of its sulphate group and leads to reduce cartilage breakdown and increase synthesis of new matrix with increase content of both proteoglycan and collagen. How these effects are achieved is unknown, but several possible modes of action have been suggested. These include reduction of superoxide radical generation, protection of chondrocytes, increased synthesis and decreased breakdown of hyaluronic acid, prevention of PGE_2 synthesis, and inhibition of a wide range of lysosomal and non-lysosomal enzymes (e.g. collagenase, cathepsins and metalloproteinases). Inhibition of proteolytic enzymes (including metalloproteinases such as stromelysin) which degrade proteoglycans may be of particular importance. High dosages of PSGAG may inhibit clotting mechanisms because of its structural resemblance to heparin.

Pharmacological effects

Polysulphated glycosaminoglycan when used by intra-articular (preferably) and IM routes slows cartilage breakdown and promotes new cartilage synthesis. In joint tissues, PSGAG prevents or reduces depressed connective tissue flexibility, resistance to compression and resiliency. The PSGAG also reduces inflammation and helps to restore synovial fluid viscosity.

It has a disease modifying effect on cartilage homeostasis as observed in models of osteoarthritis.

Pharmacokinetics

Polysulphated glycosaminoglycan is deposited in all layers of articular cartilage and is preferentially taken up by osteoarthritic cartilage following injection into joints. When administered IM, articular levels with time exceed those found in the serum. Peak joint levels are reached at about 48 hours after IM injection and persist for up to 96 hours. Following a single injection of PSGAG in horses with osteochondral defects, the drug may be detected in serum, synovial fluid and articular cartilage.

Side effects/Adverse effects

Side effects related to PSGAG seem to be minimal when using the IM route. Intra-articular administration may cause a post-injection inflammation with joint pain, effusion, swelling and associated lameness. These effects, however, appear secondary to sensitivity reactions, faulty injection technique, overdosage and frequency of injections. Adverse effects are unlikely when PSGAG is used by IM route. PSGAG has heparinoid activity and, thus, increases the activated partial thromboplastin, prothrombin, activated clotting and bleeding times when administered to dogs, cats and ponies.

Treatment: Post-intra-articular injection inflammation should be treated with anti-inflammatory drugs and cold hydrotherapy.

Contraindications and precautions

Administration of PSGAG is contraindicated in animals with a history of hypersensitivity, in shock, or with bleeding tendencies. Polysulphated glycosaminoglycan and other chondroprotective agents should not be injected into acutely inflamed or infected joints. Careful aseptic precautions should be taken when PSGAG is administered intra-articularly.

Drug interactions

While specific drug interactions have not been mentioned, using PSGAG in conjunction with either steroids or NSAIDs could mask the signs and symptoms associated with septic joints. There is some concern that since PSGAG is a heparin analogue that it should not be used in conjunction with other NSAIDs or other anticoagulants.

Clinical uses

Polysulphated glycosaminoglycan administered either by intramuscular or intra-articular route is indicated for the treatment of non-infectious and non-immune mediated arthritides, and/or traumatic joint dysfunction and associated lameness in horses. Administration of PSGAG is a useful adjunctive treatment for osteoarthritis in dogs and cats. It is also approved for the control of signs associated with traumatic (but not septic) arthritis in dogs.

Dose

Horses : 250 mg/joint, intra-articularly, repeated 4 times at weekly intervals. Joint area should be shaved and cleansed prior to injection.

: 500 mg (total dose), IM, repeated 6 times at 4 days intervals.

Dogs & cats : 1 – 5 mg/kg, IM, every 4 days for six doses, then as needed.

Pentosan polysulphate (PPS)

Pentosan polysulphate (PPS) sodium is a chondroprotective and disease-modifying agent, which has pharmacological properties similar to those of polysulphated glycosaminoglycan. Unlike PSGAG and HA, the PPS is not derived from animal source but is semi-synthetically prepared. The backbone of PPS is isolated from beech wood hemicellulose and consists of repeating units of xylanopyranoses which are sulphated synthetically. It has an average molecular weight of 5700 Da.

Most of the effects of PPS on articular cartilage and synovial joints are similar to that of PSGAG. It modulates cytokine action and preserves proteoglycan content in animal models of osteoarthritis. It possesses antithrombotic and fibrinolytic effects and may improve subchondral and synovial membrane blood flow in osteoarthritis. It has been used widely for treatment of thrombosis and interstitial cystitis (painful bladder syndrome) in humans, but its application in the treatment of arthritides is recent. In veterinary medicine, PPS is used in osteoarthritis and non-immune arthritides by intra-articular injection in horses and by intra-articular or subcutaneous injection in dogs.

Dose

Horses : 250 mg/joint, intra-articularly, repeated after 7 to 10 days.

Dogs : 10 – 20 mg (total), intra-articularly.

: 3 mg/kg, SC, repeated 3 times at 5 to 7 day intervals.

Other glycosaminoglycans

Some other glycosaminoglycans used as chondroprotective and disease-modifying agents include orally administered glycoflex and cosequin. These oral glycosaminoglycans are basically nutritional supplements which are thought to have the same effects as parenteral chondroprotective and disease modifying glycosaminoglycans like PSGAG, PPS and HA. These agents contain precursors for synthesis of hyaline cartilage matrix. Glycoflex is an extract from the *Perna canaliculus* mollusc exoskeleton, which contains glucosamine and chondroitin sulphate. Cosequin contains purified glucosamine, chondroitin sulphate and manganese ascorbate. Although these orally administered glycosaminoglycans have been found to safe, cross GI tract, and modify painful clinical signs of human and animal arthritides, their clinical use is limited primarily due to insufficient data on their efficacy and long-term adverse effects.

4. Orgotein/Superoxide dismutase

Orgotein is commercial form of Cu-Zn superoxide dismutase, a metalloprotein enzyme obtained from bovine liver. In its natural form, superoxide dismutase is complexed with copper, zinc or manganese and is found within mammalian cells, especially those of the liver. During its normal physiological action or when used as a drug, superoxide dismutase stabilises phagocyte cell membranes and lysosomes and catalyses conversion of superoxide free radicals into molecular oxygen and hydrogen peroxide (acts as free radical scavenger). Although hydrogen peroxide is pro-inflammatory, it is converted, in turn, by endogenous catalase into molecular oxygen and water. Orgotein when given intra-articularly also preserve or restore the viscosity of synovial fluid possibly by decreasing the activity of neutrophils.

Orgotein has been used to treat inflammatory problems of joints, tendons and soft tissues in horses and dogs. It can be

administered by any parenteral route for the treatment of joint inflammation, but the most effective route is intra-articular. Toxicity of orgotein has not been seen during normal course of treatment.

Dose

Horses : 5 mg (total), IM on alternate day for at least 10 days.

: 5 mg (total), intra-articularly, 1 to 4 times within a 7-day period.

Dogs : 5 mg (total), SC, once daily for 6 days. This is followed by alternate day therapy for 8 days.

5. Gold Compounds

Gold is employed in man to suppress symptoms and to retard degenerative changes in rheumatoid and other immune mediated arthritides, and is often grouped in disease modifying agents. Some oral (e.g. auranofin) and parenteral (e.g. aurothioglucose and sodium aurothiomalate) organic gold preparations have also been used to treat immune-based arthritides in dogs but the slow onset of action, difficulty in assessing efficacy, potential toxicity to haematopoietic system, and high cost have restricted their veterinary use. The application of gold compounds to medicine is called "chrysotherapy" and "aurotherapy."

Auranofin

Auranofin is an orally administered organogold compound that contains about 29% gold. It is very less soluble in water and soluble in alcohol.

Mechanism of action

The exact mechanism of action of auranofin is not well understood. On administration, gold is taken up by macrophages where it inhibits phagocytosis and may inhibit lysosomal enzyme activity. It also inhibits the release of histamine and the production of prostaglandins. Auranofin is reported to suppress helper T-cells, without affecting suppressor T-cell population. Gold has high affinity for sulphur, so the inhibitory action on sulphydryl systems may also be involved in pharmacological effects.

Pharmacological effects

Auranofin possesses anti-inflammatory, antirheumatic, immunomodulatory and antimicrobial (*in vitro*) effects. It often arrests the progress of joint lesions but its effect is slow and gradual; favourable responses may not be seen until 5-6 months after beginning therapy. It has little or no anti-inflammatory action in other circumstances. Auranofin is less efficacious than injectable gold preparations.

Pharmacokinetics

Auranofin is absorbed to the extent of 20 to 25 percent after oral administration. It is moderately bound to plasma proteins and tissues and does not accumulate in body. It crosses placenta and also distributes into milk. Auranofin is excreted in the urine and also in faeces. Unabsorbed auranofin is excreted in the faeces.

Side effects/Adverse effect

All gold preparations are relatively toxic. A dose-related immune-mediated thrombocytopenia, haemolytic anaemia or leucopenia has been noted in dogs. It has high incidence of dose-dependent GI disturbances, particularly diarrhoea in dogs. Renal toxicity or hepatotoxicity may also occur but is less common than haematotoxicity or GI disturbances.

Treatment: Discontinuation of auranofin therapy often resolves the adverse effects. Use of chelating agents (e.g. penicillamine and dimercaprol) for severe gold toxicity has been recommended, but their use is controversial.

Contraindications and precautions

Auranofin should be administered to dogs only where other less expensive and less toxic therapies are ineffective. It has been reported to be teratogenic and maternotoxic and hence should not be used in pregnancy. Urine-protein concentrations and blood cell counts should be monitored regularly in treated animals

Drug interactions

Concomitant use of gold salts with penicillamine, chloroquine or other disease modifying agents is not recommended due to increased potential for haematological or renal toxicity.

Clinical uses

Auranofin has been used to treat idiopathic polyarthritis and pemphigus foliaceus in dogs. Auranofin and other gold salts are particularly more useful in immune-based arthritides, but should be reserved for patients with progressive disease that do not respond satisfactorily to therapy with NSAIDs.

Dose

For immune-mediated arthropathies and dermatopathies

Dogs : 0.05 – 0.2 mg/kg, PO, 2 times daily. Maximal daily dose should not exceed 9 mg (total dose). It may be used in combination with prednisolone (0.5 mg/kg).

Aurothiomalate sodium

Aurothiomalate sodium (sodium aurothiomalate, gold sodium thiomalate) is a water-soluble gold compound, which possesses anti-inflammatory, antirheumatic and immunomodulatory activities. Although several actions of aurothiomalate sodium are similar to those of auranofin, it is used parenterally (IM) due to its erratic absorption from the GI tract. After IM injection, it is absorbed well and distributed to several tissues; highest concentrations are found in the synovial fluid. However, there appears to be no correlation with serum levels and efficacy as it usually takes few weeks for a beneficial effect to be noted after beginning of therapy. Aurothiomalate sodium is relatively more toxic (haematopoietic system toxicity) than auranofin. Like auranofin, it is used primarily for immune-based arthritides. The treatment regimens consist of weekly IM injections for up to 13 weeks.

Dose

Dogs : 10 mg, IM, followed by 6 injections of 20 mg, and then 6 injections of 50 mg, at weekly intervals for over 13 weeks.

Horses & cattle: 10 mg, IM, followed by 1 injection of 20 mg, then 2 injections of 50 mg, and then 9 injections of 100 mg at weekly intervals for over 13 weeks.

Note: Dosage of aurothiomalate sodium can be modified according to severity of the condition and size and breed of the animal treated.

Aurothioglucose

Aurothioglucose, also known as gold thioglucose, is a water-soluble parenteral gold compound. It contains approximately 50% gold and possesses actions like aurothiomalate sodium. In human medicine, it is used primarily in the treatment of rheumatoid

arthritis. In veterinary medicine, aurothioglucose is generally used for treating the immune-mediated serious skin disorders such as pemphigus complex. Aurothioglucose has an interaction with the antimalarial drug hydroxychloroquine.

6. Miscellaneous Anti-Inflammatory Agents

Cytotoxic immunosuppressants

Cytotoxic drugs with immunosuppressant properties such as cyclophosphamide, methotrexate, azathioprine, chlorambucil and 6-mercaptopurine have been used in the treatment of immune-based arthritides mainly in dogs. These drugs are generally given in combination with corticosteroids such as prednisolone. The cytotoxic drugs are potentially toxic and must be used with care. The detailed pharmacology of cytotoxic immunosuppressants has been given in Chapter 57.

Copper-containing compounds

Copper and copper containing compounds are reported to suppress inflammatory reactions probably through scavenging free radicals, induction of superoxide dismutase or ceruloplasmin, and reduced synthesis of prostaglandins. Some products also contain NSAIDs (e.g. copper salicylate and copper phenylbutazone) which have been claimed to have greater anti-inflammatory and lesser toxicity than the parent NSAID. The therapeutic value of copper-containing compounds in joint diseases, however, remains to be established.

Antimalarial drugs

Chloroquine and its congener hydroxychloroquine possess activity against rheumatoid arthritis and are used occasionally in human medicine. Their effects become evident only after several weeks or months of treatment. The antimalarial drugs are reserved for patients those are refractory to traditional therapeutic regimens (e.g. NSAIDs) because the long-term use of these drugs has been associated with irreparable corneal damage and ocular toxicity.

Penicillamine

Penicillamine is an effective chelator of metals (e.g. copper, mercury, zinc and lead) and is used therapeutically as antidote to metal poisoning (See for detail Chapter 76). Penicillamine is used occasionally as an alternative to gold in the treatment of rheumatoid arthritis in human practice. Although its mechanism of action in rheumatoid arthritis remains uncertain, suppression of disease has been related to reduction in concentration of IgM rheumatoid factor. Penicillamine has slow onset of action and is more likely to produce serious toxicity, including blood dyscrasias, cutaneous lesions and a number of autoimmune disorders.

Tetracyclines

The tetracycline antibiotics are broad-spectrum bacteriostatic agents which are believed to have a disease modifying effect in both rheumatoid arthritis and osteoarthritis. Tetracyclines, especially doxycycline and minocycline, are reported to have ability to inhibit the activity of metalloproteinases, collagenases and gelatinase *in vitro*. These drug, however, are not used clinically for the treatment of arthritides.

Leukotriene antagonists

A large number of drugs have been developed recently which block either leukotrienes formation or leukotrienes receptors. Leukotrienes (LTs) contribute to the inflammatory response through a variety of effects such as vascular permeability (LTC_4, LTD_4 and LTE_4), neutrophil aggregation, degranulation, chemotaxis (LTB_4), etc. Inhibitors of 5-lipoxygenase, the enzyme which generate leukotrienes, include piripost and zileuton. An LTD_4 antagonist, zafirlukast has shown promise in the treatment of asthma. Human studies have shown that these types of agents have efficacy in the treatment of various types of asthma and other inflammatory conditions.

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SECTION-V

DRUGS ACTING ON CARDIOVASCULAR SYSTEM

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CHAPTER 31 GENERAL CONSIDERATIONS

Heart
- Physiology of cardiac functions
  - Cardiac action potential
  - Excitability
  - Refractory period
  - Cardiac conductivity
  - Cardiac cycle
  - Electrocardiogram (ECG)
  - Excitation-contraction coupling
  - Maintenance of electrochemical gradient
  - Regulation of cardiac pumping

Blood pressure
- Types of blood pressure
- Control of blood pressure

The cardiovascular system is an important organ system that circulates blood, oxygen and nutrients in the body. It comprises of three major components: heart, blood vessels and blood. The cardiovascular systems of mammals are closed, meaning that the blood never leaves the heart and network of blood vessels. In contrast, oxygen and nutrients diffuse across the blood vessel layers and enters interstitial fluid, which carries oxygen and nutrients to the target cells, and carbon dioxide and wastes in the opposite direction to the blood vessels and then to the heart. The three components of cardiovascular system are, therefore, not independent, but interlinked with each other. Drugs acting on the cardiovascular system have their major action on either one or more of these three components of cardiovascular system.

HEART

The heart is an important organ that pumps and propels oxygenated blood to the body and deoxygenated blood to the lungs. Impairment of any cardiac function inevitably leads to significant dysfunction and loss of health. Accordingly, a practical understanding of cardiac function and dysfunction is important for proper comprehension of various pharmacological agents.

PHYSIOLOGY OF CARDIAC FUNCTIONS

The mammalian heart is a four-chambered muscular organ that is composed of three major types of cardiac muscle - atrial muscle, ventricular muscle, and specialised excitatory and conductive muscle. The atrial and ventricular types of cardiac muscle contract in much the same way as skeletal muscle, but the specialised excitatory and conductive fibres contract only feebly because they contain few contractile fibres. Instead, these fibres exhibit rhythmicity and varying rates of conduction providing an excitatory system for the heart.

Cardiac Action Potential

The cardiac action potential is a specialized action potential in the heart, necessary for the electrical conduction system of the heart. Electrophysiologically, heart consists of two types of muscle fibre – the non-automatic fibre that shows fast channel action potential, and the automatic fibre that shows slow channel action potential. The shape and duration of each action potential are determined by the activity of ion channels present on surface of individual cells.

Non-automatic fibres: Non-automatic fibres are ordinary cardiac muscle fibres of atria, ventricles and also Purkinje fibres, which do not possess property of spontaneous depolarisation and hence cannot generate an impulse of their own. Instead they are excited to depolarise when they receive the propagated action potential from nodal or pacemaker cells, or other cells of specialised conduction system. Unlike a skeletal muscle that has only Na^+ channels, both Na^+ and Ca^{++} channels cause the action potential in non-automatic cardiac muscle. The Na^+ channels are fast channels because they open quickly and close quickly (~ 1/10 ms), while the Ca^{++} channels are slower to open and also remain open for longer period (~ 200 ms). Each action potential in non-automatic ventricular and atrial muscle cells and Purkinje fibre is divided into 5 phases viz. 0, 1, 2, 3 and 4. The standard model used to understand the cardiac action potential is the action potential of the ventricular myocyte (Figure 31.1).

a. *Ventricular muscle cells:* In ventricular cells, the resting membrane potential during diastole is maintained at –80 to –90 mV (negative inside in relation to outside the cell) mainly due to uneven distribution of cations i.e. high K^+_i relative to K^+_o and low Na^+_i than Na^+_o. This gradient is established by pumps, especially Na^+-K^+-ATPase. The Na^+ (also Ca^{++}) channels are mostly closed at rest and K^+ channels are mostly open, so there is selective outward leakage of K^+ in response to concentration gradient. Once the cell is electrically stimulated (arrival of impulse), there is sudden opening of large number of Na^+ channels which allow rapid influx of extracellular Na^+. This causes the cell membrane to become positively charged on its inside surface with considerable overshoot (the positive portion of spike) causing very rapid depolarisation (Phase 0). The Na^+ channels close again very quickly and become inactivated, and the membrane begins to repolarise through opening of K^+ channels

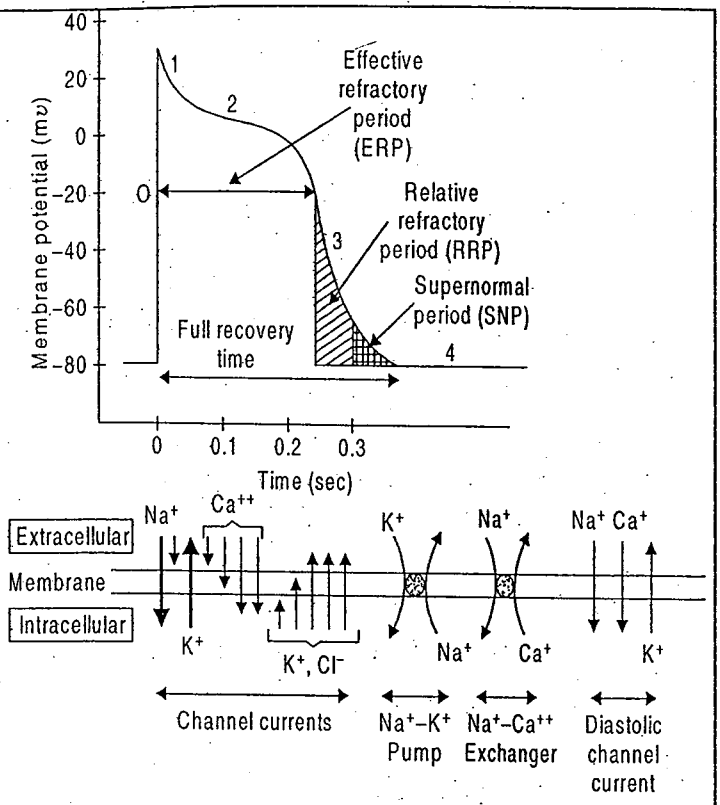


Fig. 31.1. Action potential from a typical ventricular muscle fibre showing different phases (0 to 4) and refractory periods. Also shown are various ion-channel currents.
Phase 0 = rapid depolarisation; Phase 1 = initial repolarisation; Phase 2 = plateau; Phase 3 = repolarisation/late repolarisation; Phase 4 = resting membrane potential.

(Phase 1 or the partial repolarisation). This repolarisation, however, is interrupted by opening of slow Ca^{++} channels which result in a prolonged plateau of depolarisation (Phase 2). The plateau is facilitated by closing of many K^+ channels in response to opening of Ca^{++} channels, which decrease K^+ efflux (Figure 31.2). After about 200 ms (plateau time), the Ca^{++} channels close and the K^+ channels reopen causing the cell to repolarise (Phase 3) and eventually to reach the stable, negative resting membrane potential (Phase 4). Resting membrane potential once attained, does not decay till opening of Na^+ channels and start of next action potential.

b. Atrial muscle cells: The action potential in the atrial muscle cells is basically similar to those of ventricular cells except that atrial slow Ca^{++} channels open for shorter time and atrial K^+ channels close for a shorter time. As a result, the plateau of an atrial cell's action potential is shorter and not as 'flat' as the plateau of a ventricular cell's action potential. Therefore, atrial muscle cells have a shorter action potential and also shorter refractory period (Figure 31.2), which allow more action potential (beats) per minute than ventricular cells.

Automatic fibres: Automatic fibres are present mainly in the sinoatrial (SA) and atrioventricular (AV) nodes. In addition, patches of automatic tissue are present in His-Purkinje system, interatrial septum, AV-ring and around opening of large vein, which can acquire automaticity (pacemaker activity) under exceptional or pathological conditions.

a. Sinoatrial node (SA node): The cardiac pacemaker cells of SA node exhibit an intrinsic rhythmicity (automaticity or pacemaker activity) that is independent of external nervous or hormonal activity. The resting membrane potential of SA nodal fibre is –55 to –60 mV in comparison with –85 to –95 mV for ventricular fibre. The cardiac pacemaker cells of SA node lack voltage gated fast Na^+ channels (some scientists suggest that fast Na^+ channels are present but are inactivated at resting potential of –55 to –60 mV), therefore, nodal fibres do not show the rapid depolarisation (Phase 0) seen in ventricular and atrial cells. Instead, these cells have pacemaker Na^+ channels that close during an action potential but begin to open spontaneously once resting potential at –55 to –60 mV is reached. The spontaneous opening of these pacemaker Na^+ channels causes a progressive influx of Na^+, which slowly depolarizes the cell towards threshold called the spontaneous depolarization or pacemaker potential. At the same time, K^+ channels those are open at the end of action potential begin to close, thereby decreasing K^+ permeability. Late in the spontaneous depolarization, slow Ca^{++} channels also begin to open and some Ca^{++} enter into the cell; this speeds the spontaneous depolarization to approach the threshold. Thus, spontaneous depolarisation is caused by opening of pacemaker Na^+ channels (mainly), closing of K^+ channels, and also opening of Ca^{++} channels. These changes in pacemaker cells are in contrast to the stable status of the ion channels in normal resting ventricular or atrial cells. Once threshold is reached at about –45 mV, many of the slow Ca^{++} channels (also some Na^+ channels) become activated and open leading to rapid influx of Ca^{++} (also some Na^+) and action potential ensues.

In pacemaker cells, the initiation of action potential occurs at higher threshold (less negative value) and is automatic. The overshoot of action potential is also quite slow and of low amplitude. Further, the action potential is of shorter duration and phases 1 - 3 are not clearly demarcated (Figure 31.2). The action potential in pacemaker cells is often called slow action potential because it lacks a rapid depolarisation (Phase 0), has a rounded appearance, and is caused by slow Ca^{++} channels.

Normally, the SA node has the steepest Phase 4 spontaneous depolarisation, undergoes self-excitation and propagates impulse to rest of the heart. Therefore, SA node acts as the pacemaker of heart.

b. Atrioventricular node (AV node): Similar to SA node, cells of the AV node exhibit pacemaker activity (spontaneous depolarization towards threshold) and slow action potential. However, the spontaneous depolarization in AV node cells is much slower than SA node cells (Figure 31.2). Therefore, SA node receives the propagated impulse from SA node when its cells are depolarising spontaneously and have not reached the threshold. Accordingly under normal conditions, impulse originates from SA node and helps cells of AV node to reach threshold and the pacemaker activity of AV node cells

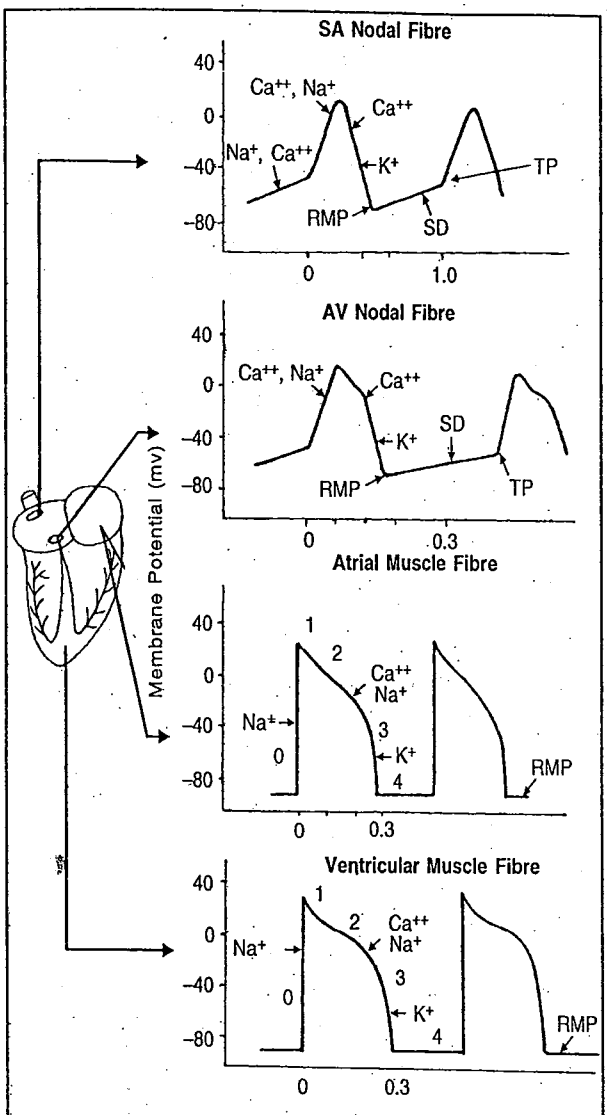


Fig. 31.2. Action potential from automatic (SA node and AV node) and non-automatic (atrium and ventricle) muscle fibres. Time and membrane potential are not strictly to the scale.

Abbreviations: RMP = Resting membrane potential; SD = spontaneous depolarisation; TP = threshold potential.

become irrelevant (spontaneous activity remains latent). However under certain abnormal conditions (e.g. damaged SA node or when SA node impulse fails to reach AV node), the AV pacemaker cells continue to depolarise spontaneously to threshold and can initiate cardiac action potential.

c. *Other cells:* Other automatic fibres also undergo Phase 4 depolarisation (automaticity), but at a much slower rate. They receive a propagated impulse before spontaneous depolarisation reaches the threshold and remain as latent pacemaker.

Excitability

Excitability of a cardiac muscle is defined by the strength of stimulus required to elicit a response or to produce an action potential. Under normal conditions, excitability is determined by the level of membrane potential (threshold level) at which cardiac cell can generate an action potential. Hyperpolarisation decreases the excitability, whereas reduction in resting membrane potential increases the excitability by increasing and decreasing the gap between resting membrane potential and threshold potential, respectively. Therefore in fast channel fibres, excitability is generally super-normal at the end of Phase 3 in which stimuli even slightly smaller than the threshold stimuli elicit a propagated response (see also refractory period below).

Refractory Period

Refractory period is the time immediately after beginning of an action potential during which cardiac muscle cell is refractory to re-stimulation i.e. another action potential cannot occur. The duration and type of refractoriness (inability of the cardiac cell to be re-excited) varies with the type of cardiac muscle.

Non-automatic fibres: In non-automatic fibres or fast response cells (e.g. atrial, ventricular and Purkinje fibre cells), the refractoriness is determined by the voltage dependent recovery of Na^+ channels from inactivation. Under normal conditions, the refractoriness begins when Na^+ channels close and become inactivated (after phase 0), and continues till the cell membrane potential returns to its resting level and the Na^+ channels inactivation ends. This total period of refractoriness (sometimes called functional refractory period or full recovery time) consists of effective refractory period (ERP), relative refractory period (RRP) and supernormal period (SNP).

The ERP lasts from the onset of depolarisation until cardiac muscle cell has partly repolarised to a membrane potential of about –50 mV. The ERP represents the true refractoriness because no Na^+ channel is open and available for excitation and the cardiac muscle cell is unresponsive to even a strong stimulus. The ERP is followed by the RRP (Figure 31.1), in which muscle is difficult than normal to excite, but nevertheless excited by an abnormally strong stimulus. The RRP depends on the number of Na^+ channels which have recovered from inactivation (a small number of Na^+ channels recover from inactivation during RRP). The RRP is followed by the supernormal period at the end of Phase 3 in which even stimuli slightly smaller than the threshold stimuli elicit a propagated response. When membrane potential returns to resting level, the cardiac muscle cell responds to normal stimulus resulting in an action potential. The RRP and SNP are not important under normal conditions, but may result in conductional abnormalities if abnormal electrical stimuli are delivered during this period. Normally in fast response cell, the refractory period/full recovery time lasts about as along as an action potential.

Automatic fibres: Regions which lack fast Na^+ channels (automatic fibres) have much longer refractory period than the fast conducting tissues. In Ca^{++} channel dependent tissues (e.g. AV node), the recovery from slow inward current inactivation is slow and is time dependent. In such tissues, even after an action potential has repolarised back to its initial resting potential, Ca^{++} channels are not all available for re-excitation. Therefore in slow response tissues, the refractory period outlasts the normal action potential. The longer refractory period in such tissues is advantageous to body. The long refractory periods of AV node cells prevent the normal action potential from 'circling back' and reactivating atria once it reaches the ventricle. Similarly, longer refractory period of AV node cells protect the ventricles from over stimulation, especially in conditions like atrial fibrillation or atrial flutter in which atrial action potentials are extremely frequent.

Cardiac Conductivity

The SA node, AV node, AV bundle, bundle branches and Purkinje fibres are collectively called the specialised conduction system of heart, because they are composed of specialised cardiac muscle fibres responsible for the initiation and organisation of cardiac contractions. The cardiac impulse originate from the SA node and spreads rapidly across right and left atria causing both atria to contract. Impulse is carried also from the SA node to AV node through the internodal pathways. From the AV node, impulse is carried onto the AV bundle or bundle of His, which are divided into the two right and left bundle branches. These bundle branches break up at the ventricular apex into a network of fine fibres, the Purkinje fibres, and turn back towards the base of heart (Figure 31.3). The Purkinje fibres make contact with all muscle fibres of the ventricle and then become continuous with the cardiac muscle fibre.

The conduction velocity at which impulses are propagated through conduction system and myocardium is a function of the amplitude of action potential (dv) and its rate of rise (dt), expressed as dv/dt of phase 0 or membrane responsiveness. Both dv and dt are related to the membrane potential present at the onset of action potential. A smaller (less negative) membrane potential (e.g. AV node) results in decrease in amplitude of action potential and slope of phase 0 and, thus, a decrease in conduction velocity. On the other hand, a more polarised membrane (e.g. atrial, ventricular and Purkinje fibres) depolarises faster and show faster conduction velocity. Therefore, the conduction velocity is lowest in AV node (0.05 m/sec) and fastest in Purkinje fibres (1.5 to 4 m/sec).

Normally, an impulse originates in the SA node (0.0 second in Figure 31.3) and spreads within 0.1 second at moderate velocity (0.3 m/second) through the right and left atria. The impulse from SA node also reaches first cells in the AV node (0.03 m/second) and propagates very slowly. It is delayed for about 0.13 second (0.16 second minus 0.03 second) before appearing in the ventricular septal AV bundle. Slow conduction through AV junction creates the delay between atrial and ventricular contractions as it provides the only route for conduction of action potentials from atria to ventricles (Figures 31.3). Once the impulse has entered the AV bundles, it spreads rapidly through Purkinje fibres to the entire endocardial surfaces of both ventricles. Then the impulse once again spreads at moderate speed (0.3 to 0.5 m/second) through the ventricular muscle to epicardial surfaces. The ventricular polarisation is complete by 0.22 seconds and both ventricles contact. By this time, the atria have repolarised to a resting state and are relaxing. The conducted impulse dies out after it has activated the ventricles because it is surrounded by refractory tissue that it has just excited.

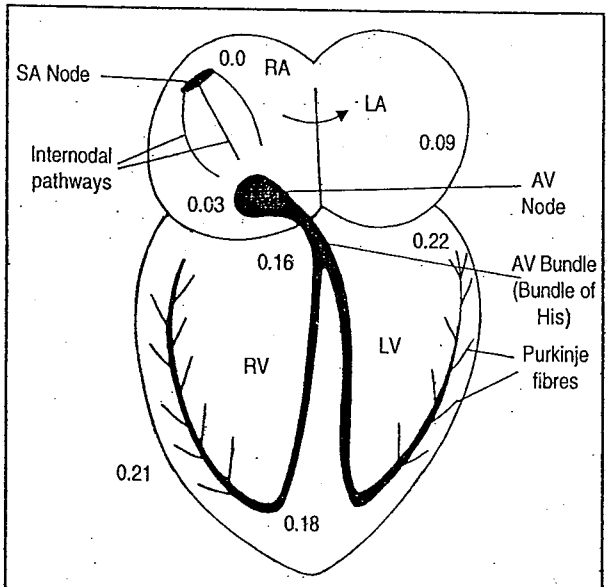


Fig. 31.3. Schematic representation of initiation and transmission of cardiac impulse in the heart. The time of appearance of impulse in different parts of heart is shown also (in fractions of a second).

Abbreviations : RA = right atrium ; LA = left atrium ; RV = right ventricle; LV = left ventricle.

Cardiac Cycle

The conduction events that occur from the beginning of one heart beat to the next are called the cardiac cycle. The cardiac cycle consists of a period of relaxation called diastole, during which the heart fills with blood, followed by a period of contraction called systole. Normally, cardiac muscle begins to contract a few milliseconds after the action potential begins and continues to contract until a few milliseconds after the action potential ends. Therefore, the duration of contraction is mainly a function of the duration of action potential. In a normal beat, both atria contract almost simultaneously followed by a brief pause (caused by slow conduction through AV node), and then two ventricles contract almost simultaneously. The delay of more than 1/10 second between passages of cardiac impulse from atria into ventricles allows the atria to contract ahead of ventricles, thereby pumping blood into the ventricles. After ventricular contraction, the heart relaxes and refills and remains in a resting state until the SA node pacemaker cells originate next beat.

Electrocardiogram (ECG)

The electrocardiogram (ECG) is the reading of electrical voltage (electrical activity) generated by heart, which is recorded by the electrocardiograph from surface of the body. The normal electrocardiogram (Figure 31.4) is composed of a P wave, a QRS complex and a T wave. The P wave corresponds to atrial depolarisation, the QRS complex indicates ventricular depolarisation and the T wave records ventricular repolarisation. The PR interval gives the time taken for impulses to travel from SA node to ventricular muscle. The QRS complex starts with arrival of impulse at the AV node and ends with activation of the Purkinje fibres in ventricular tissue. The Q-T interval indicates ventricular systole. The time between successive P waves (PP interval) corresponds to the time between atrial contractions, and RR interval corresponds to the time between ventricular contractions.

Electrophysiologically, the P wave and the components QRS complex are called depolarisation waves and the T wave is known as a repolarisation wave. The depolarisation of SA node and AV node is not recorded on conventional ECG because their small mass is inadequate to produce recordable surface potential. The atrial repolarisation is usually obscured by the much larger QRS wave; therefore, atrial depolarisation is not observed also with electrocardiogram.

Electrocardiography is an important diagnostic and prognostic tool for the detection and management of cardiac abnormalities. Prolongation of P-R interval indicates an abnormality of the conduction system. The Q-T interval becomes shorter with

Table 31.1: Characteristics of fast channel and slow channel potentials.

Sr. No.	Feature	Fast channel action potential	Slow channel action potential
1.	Site of occurrence	Atria, ventricles, Purkinje fibres	SA node, AV node, interatrial septum, coronary sinus opening.
2.	Predominant ions responsible for depolarisation	Na^+ (Phase 0), Ca^{++} (Phase 2)	Ca^{++}
3.	Action potential phases (0 – 4)	Clearly demarcated	Less clearly demarcated.
4.	Activation-inactivation kinetics.	Fast	Slow
5.	Spontaneous depolarisation (Pacemaker activity)	Absent (in Purkinje fibre cells present)	Present
6.	Resting membrane potential	–80 to –90 mV	–55 to –60 mV
7.	Activation potential (threshold voltage)	–60 to –90 mV	–40 to –45 mV
8.	Conduction velocity	0.5 to 5 m/second	0.01 to 0.1 m/second
9.	Overshoot	+20 to +35 mV	0 to +15 mV
10.	Refractory period-action potential relationship	RP = APD (ERP < APD)	RP > APD (ERP > APD)
11.	Relationship to nodal tissues	Probably none	May mediate pacemaker potentials.
12.	Selective channel blocker	Tetrodotoxin, local anaesthetics	Verapamil, Mn^{++}

Abbreviations: SA = sinoatrial node; AV node = atrioventricular node; RP = refractory period; ERP = effective refractory period; APD = action potential duration.

increasing heart rate. In cardiac arrhythmias, the normal ECG values are altered.

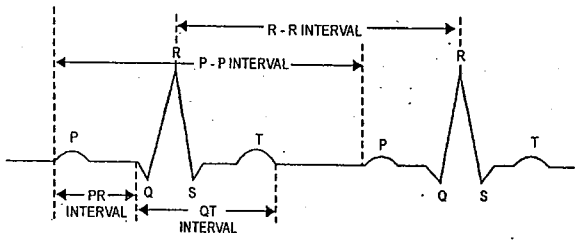


Fig. 31.4. Normal Electrocardiogram

Excitation-Contraction Coupling

The term 'excitation-contraction coupling' means the physiological mechanism by which the action potential causes the myofibrils of muscle to contract. In cardiac muscle, the method is dependent on a phenomenon called calcium-induced calcium release, which involves the conduction of calcium ions into the cell triggering further release of ions into the cytoplasm. In this process, an action potential is first induced by pacemaker cells in the SA node and conducted from non-contractile cardiac myocytes to cardiac contractile cells. This triggers L-type calcium channels during the plateau phase of the cardiac action potential, causing a net flux of calcium ions into the cardiac myocyte (electrogenic pathway). Although this concentration of calcium is not sufficient to activate contractile apparatus, it triggers a large release of additional Ca^{++} from intracellular storage sites of the sarcoplasmic reticulum (also mitochondria) in a positive feedback physiological response (Figure 31.5). This process is called calcium-triggered calcium release. Release of calcium from sarcoplasmic reticulum also occurs when the action potential spreads to interior of the cardiac muscle fibre along with membranes of the transverse (T) tubules. There is also a non-electrogenic pathway in which Ca^{++} enters through Na^+-Ca^{++} exchange i.e. in response to phase 0 entry of Na^+, the Na^+-Ca^{++} exchange protein may transiently extrude three Na^+ ions in exchange for one Ca^{++} ion. These calcium sources (mainly electrogenic and perhaps non-electrogenic) increase the intracellular calcium concentrations by some 100 fold (from 10^{-7} M to 10^{-5} M), which activate the contractile proteins. At this stage, high Ca^{++} binds to high affinity subunits of troponin molecule and catalyses chemical reactions which promote sliding of actin and myosin filaments along one another causing the muscle contraction.

Relaxation of cardiac muscle occurs as the Ca^{++} moves from troponin binding sites and the free cytosolic calcium is pumped back into sarcoplasmic reticulum and mitochondria or pumped back across the cell membrane into extracellular fluid through an electrogenic Na^+-Ca^{++} exchanger (Figure 31.5). Both these processes involve active transport because the Ca^{++} is being pumped against its electrochemical gradient. The Na^+-Ca^{++} exchange mechanism on the cell surface exchanges three Na^+ ions from the exterior for each Ca^{++} ion extruded. These processes decrease the cytosolic Ca^{++} concentrations to its resting level and the cardiac muscle relaxes.

Maintenance of Electrochemical Gradient

With each action potential, there is net influx of Na^+ and Ca^{++}, and efflux of K^+. Some membrane bound enzymes act as pumps to relocate ions to prevent their improper accumulation. An ATP-requiring Na^+-K^+ exchange mechanism or pump (Na^+, K^+-ATPase) located in the cell membrane is activated in most cells that extrudes three Na^+ ions for every two K^+ ions into the cell against their respective concentration gradients (Figure 31.5). Excess Ca^{++} in the intracellular space is pumped out by systems localised in regions of sarcoplasmic reticulum by Ca^{++}-ATPase and Na^+-Ca^{++} exchanger as mentioned above in the excitation-contraction coupling.

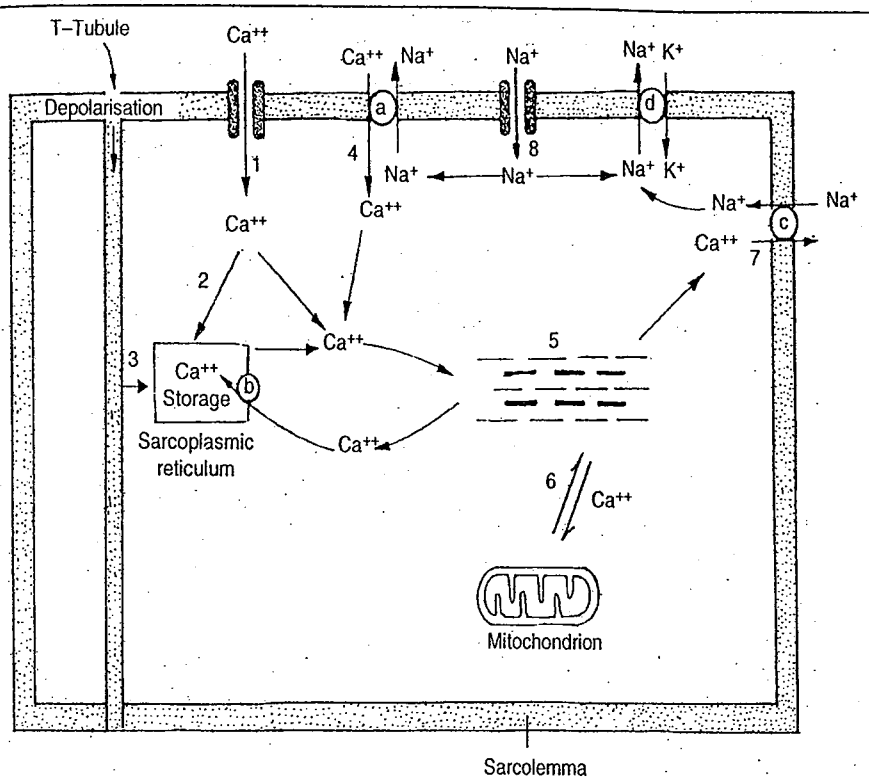


Fig. 31.5. Schematic representation of electrolyte maintenance and excitation-contraction in cardiac cell.

1. Ca^{++} enters cardiac cell through voltage sensitive Ca^{++} channel.
2. Inward moving Ca^{++} triggers a large release of Ca^{++} from intracellular storage site of sarcoplasmic reticulum.
3. Spread of action potential along membrane of T-tubule also releases Ca^{++} from sarcoplasmic reticulum.
4. Ca^{++} enters also the cardiac cell through $Na^+ - Ca^{++}$ exchanger (a).
5. Enhanced intracellular Ca^+ from various sources causes muscle contraction.
6. Free cytosolic Ca^{++} after muscle contraction is pumped back into sarcoplasmic reticulum and mitochondria by an active Ca^{++} – ATPase (b).
7. Free cytosolic Ca^{++} is pumped out through $Na^+ - Ca^{++}$ exchanger (c).
8. Influx of Na^+ through voltage sensitive Na^+ channel.
9. Extrusion of Na^+ and intrusion of K^+ through $Na^+ - K^+$ –ATPase pump (d).

Regulation of Cardiac Pumping

The volume of blood pumped by heart per minute is termed as cardiac output. Cardiac output is a function of heart rate and stroke volume. The heart rate is the number of heart beats per minute and the stroke volume is the volume of blood, in millilitres (ml), pumped out of the heart with each beat. Increasing either heart rate or stroke volume increases cardiac output.

Cardiac output (ml/min) = Stroke volume (ml/contraction) x Heart rate (beats/min)

The amount of blood pumped by the heart each minute is regulated mainly by two mechanisms - the intrinsic regulation and the autonomic nervous system.

Intrinsic regulation of heart pumping: Under physiological conditions, heart adjusts its pumping activity by changing its own length. In heart, the volume of blood returning from veins (venous return) mainly controls the resting muscle length. When an extra amount of blood flows into heart, the cardiac muscle is stretched to accommodate it. The stretched cardiac muscles then respond with enhanced contractile strength, thus pumping excess of blood into systemic arteries so that blood can flow around the circuit again. This intrinsic ability of the heart muscle to adapt to changing volumes of inflowing blood is called the 'Frank Starling mechanism of the heart'. Basically, the Frank starling mechanism means that greater the heart muscle is stretched during filling, the greater will be the force of contraction and the greater will be the quantity of blood pumped into aorta. Therefore, within physiological limits, the intrinsic regulation allows heart to pump all blood that comes to it without allowing its excessive accumulation in veins.

Regulation by autonomic nervous system: The autonomic nervous system regulates the pumping effectiveness of heart by adjusting cardiac rate or myocardial contractility, or both. Sympathetic stimulation can increase both heart rate and force of contraction, thereby increasing the volume of blood pumped and also the ejection pressure. The increase in force of contraction occurs irrespective of end diastolic filling (venous return) and muscle length. Such a change in contractile strength (independent of muscle length) is called the inotropy. The intrinsic capability of heart to adjust to filling pressure persists even in the presence of inotropic stimulation by sympathetic nervous system. The cardiac output can often be increased more than 100 percent by a strong sympathetic stimulation (Figure 31.6).

In contrast to sympathetic nervous system, the parasympathetic nervous system decreases cardiac output mainly by adjusting heart rate and not by changing inotropic state. A strong vagal stimulation of heart can produce marked bradycardia with fewer heartbeats per minute, but its depressant effect on the strength of heart contraction is only marginal (up to 20-30 percent). This is

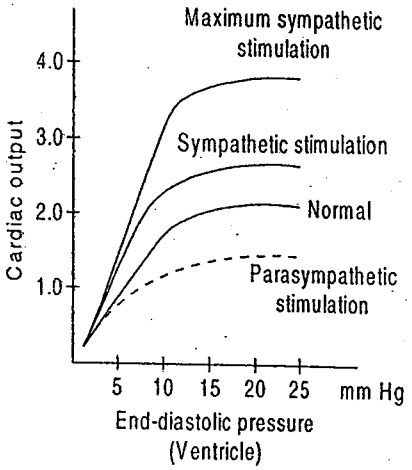


Fig. 31.6. Effect on the cardiac output curve of different degrees of sympathetic and parasympathetic stimulation.

because the vagal fibres are distributed mainly to atria and not to muscles of ventricles where the power contraction of heart occurs.

Schematic representation of control of cardiac output is given in Figure 31.7.

BLOOD PRESSURE

Blood pressure (BP) is the pressure exerted by circulating blood upon the walls of blood vessels. During each heartbeat, BP varies between a maximum (systolic) and a minimum (diastolic) pressure. The mean BP, due to pumping by the heart and resistance to flow in blood vessels, varies greatly as the circulating blood moves away from the heart through arteries into the vena cava. The blood pressure is maximum about ~ 98 mmHg in the aorta and minimum about ~ 3 mmHg in the vena cava. This pressure difference between the aorta and the vena cava forces the blood to move through systemic vessels. The decrease in the blood pressure as the blood flows through various segments of systemic circulation (from aorta to vena cava), occurs mainly due to resistance which the walls of blood vessels exert on the flow of blood. The greatest decrease in blood pressure occurs when the blood is flowing through the arterioles because they provide a greater resistance to blood flow than any other segment of systemic circulation. Although this resistance is inversely related to the diameter of blood vessels (i.e. smaller the diameter, greater the resistance), the net resistance of capillaries is less than that of arterioles because each arteriole delivers blood to a number of capillaries. The net resistance of systemic circulation (mainly arterioles) is called the total peripheral resistance (PVR) or the systemic vascular resistance (SVR). It is given by the formula

$$\text{Total peripheral resistance} = \frac{\text{Mean aortic pressure (mmHg)} - \text{Vena caval pressure (mmHg)}}{\text{Cardiac output (L / minute)}}$$

Because the pressure in vena cava is usually close to zero, it is sometimes ignored in calculating total peripheral resistance.

Types of blood pressure

Systolic and diastolic pressures: The actual blood pressure in the aorta and arteries is pulsatile, increasing with each cardiac ejection and falling in between ejections. The maximum arterial pressure resulting from ventricular contraction or systole is termed as systolic blood pressure. During the relaxation or diastolic phase, the blood pressure falls significantly but the elastic recoil of arteries avoids a steep fall. The lower blood pressure seen during relaxation of heart is called the diastolic blood pressure.

Pulse pressure: It is the pressure difference between systolic and diastolic blood pressures. The pulse pressure is determined by the interaction of the stroke volume of the heart, compliance (ability to expand) of the aorta, and the resistance to flow in the arterial tree. The pulse pressure can be simply calculated from the difference of the measured systolic and diastolic pressures.

$$P_{pulse} = P_{sys} - P_{dias}$$

A narrow or low pulse pressure can be caused by congestive heart failure, shock, aortic stenosis and significant blood loss in trauma. A consistently high pulse pressure may reflect stiffness of the major arteries, aortic regurgitation (a leak in the aortic valve), hyperthyroidism or some other conditions.

Mean arterial pressure (MAP): It is the average arterial pressure during a single cardiac cycle. The MAP is determined by the cardiac output (CO), systemic vascular resistance (SVR), and central venous pressure (CVP) according to the following relationship, which is based upon the relationship between flow, pressure and resistance:

$$MAP = (CO \cdot SVR) + CVP$$

In practice, MAP is not determined by knowing the CO and SVR, but rather by measurements of the systolic pressure (P_{sys}) and the diastolic pressure (P_{dias}), while there is a normal resting heart rate.

$$MAP = P_{dias} + 1/3\ (P_{sys} - P_{dias}) \text{ or}$$

$$\text{Mean blood pressure} \approx \text{Diastolic pressure} + \frac{\text{Pulse pressure}}{3}$$

The mean arterial pressure is considered to be the perfusion pressure seen by organs in the body. Any significant change either in the cardiac output or in the systemic vascular resistance may affect MAP. If CO and SVR change reciprocally and proportionately, then MAP will not change.

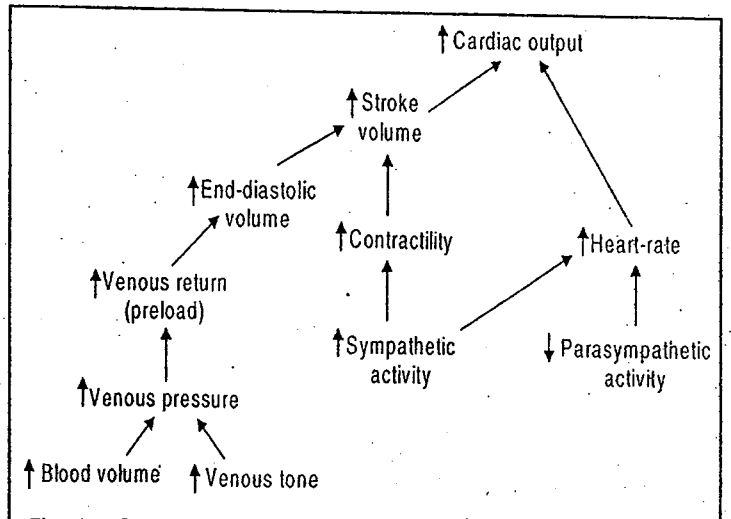


Fig. 31.7. Schematic representation of control of cardiac output.

Central venous pressure (CVP): It is the vascular pressure in the thoracic vena cava, near the right atrium of the heart. The CVP reflects the amount of blood returning to the heart and the ability of the heart to pump the blood into the arterial system. It is a good approximation of right atrial pressure. The CVP is increased by venous blood volume or by increase in venous tone. The CVP is generally increased in hypervolaemia, tension pneumothorax, forced exhalation (e.g. asthma or chronic obstructive pulmonary disease), pleural effusion and heart failure and is decreased in hypovolaemia, septic shock, deep inhalation and increased venous compliance.

Pulmonary pressure: It is the vascular pressure in the pulmonary artery. It is about 15 mmHg in human beings at rest. Increased BP in the capillaries of the lung causes pulmonary hypertension, with interstitial oedema if the pressure increases to above 20 mmHg, and to frank pulmonary oedema at pressures above 25 mmHg.

Control of Blood Pressure

The blood pressure is determined by the rate of blood flow produced by heart (cardiac output), and the resistance of blood vessels to blood flow (peripheral vascular resistance). Therefore, alteration in blood pressure occurs mainly due to change in either the cardiac output (CO) or the total peripheral resistance (PVR), or both. The blood pressure is generally given by the following formula.

Blood pressure ≈ Cardiac output x Total peripheral resistance

Using the formula, the blood pressure can be raised either by increasing CO or PVR, and reduced by a decrease in cardiac output or PVR. In normal animals, both cardiac output and peripheral vascular resistance are controlled mainly by two overlapping control mechanisms, the baroreflexes mediated by sympathetic nervous system and the renin-angiotensin-aldosterone system. The vascular tone and intravascular pressure may be elevated because of increased sympathetic tone, activation of angiotensin, release of aldosterone, or increased vascular wall resistance caused by salt and water retention.

1. ***Baroreceptor and systemic nervous system:*** Baroreceptors and systemic nervous system is responsible for the rapid moment-to-moment control of blood pressure. A fall in blood pressure depresses baroreceptor activity in the aortic arch and carotid sinus. The central nervous system responds reflexly to depressed baroreceptor activity by increasing sympathetic efferent activity to the heart and blood vessels, and by decreasing parasympathetic activity to the heart. Enhanced release of norepinephrine from sympathetic nerve fibres causes peripheral vasoconstriction (α_1- activity) and increased cardiac output (β_1-activity). This results in compensatory rise in blood pressure (Figure 31.8).
2. ***Renin-angiotensin-aldosterone system:*** Renin-angiotensin-aldosterone system is responsible for long-term control of blood pressure mainly by altering blood volume. Baroreceptors in the kidneys respond to the reduced blood pressure (and to sympathetic stimulation of β-adrenoceptors) by releasing renin, which in turn enhances synthesis of angiotensin II. Elevated angiotensin II produces peripheral vasoconstriction and enhanced secretion of aldosterone leading to sodium and water retention. All these factors contribute to a further increase in blood pressure.

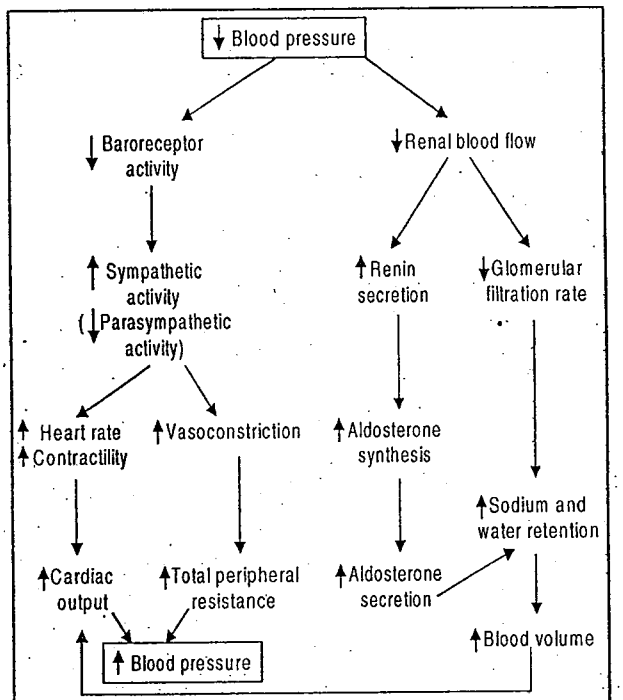


Fig. 31.8. Schematic representation of regulation of blood pressure by baroreceptor-sympathetic system and renin-angiotensin-aldosterone system.

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CHAPTER 32

MYOCARDIAL STIMULANTS

Classification
Cardiac glycosides/Digitalis
  Source and chemistry
  Mechanism of action
Phosphodiesterase inhibitors
  Methylxanthines
  Bipyridine derivatives
  Pyridazone derivatives
  Miscellaneous drugs
Beta-adrenoceptor agonists
Miscellaneous agents

Myocardial stimulants can be defined as positive inotropic drugs which enhance the strength of cardiac muscle contractility and hence increase cardiac output. These drugs are used primarily in conditions like congestive heart failure (CHF) when heart is unable to pump sufficient blood to meet the needs of the body. Congestive heart failure is usually caused by an impaired ability of the cardiac muscle to contract or by an increased workload on the heart. In dogs and cats, treatment of CHF with myocardial stimulants can significantly improve life expectancy and quality of life, but for working animals therapy is rarely economically viable.

Classification

Myocardial stimulants used in veterinary practice include many groups. Although these drugs act by different mechanisms, in each case the inotropic action is the result of an increased cytoplasmic calcium concentration that enhances contractility of cardiac muscle.

I. Cardiac glycosides
   e.g. digoxin, digitoxin and ouabain.
II. Phosphodiesterase inhibitors
   1. Methylxanthines
      e.g. aminophylline, theophylline and etamiphylline.
   2. Bipyridine derivatives
      e.g. amrinone and milrinone.
   3. Pyridazone derivatives
      e.g. pimobendan and levosimendan.
   4. Miscellaneous drugs
      e.g. enoximone.
III. Beta-adrenoceptor agonists
   e.g. epinephrine, isoprenaline, dobutamine and dopamine.
IV. Miscellaneous agents
   e.g. calcium and glucagon.

I. CARDIAC GLYCOSIDES/DIGITALIS

The cardiac glycosides are a group of chemically similar compounds which have cardiac inotropic properties. The cardiac glycosides are often called digitalis or digitalis glycosides because most of these drugs come from the digitalis (Foxglove) plant. Cardiac glycosides are used clinically in the management of congestive heart failure and to control certain cardiac arrhythmias. Commonly used cardiac glycosides include digoxin and digitoxin.

Source and Chemistry

Cardiac glycosides are present in a number of plants. A few of these compounds are also present in the venom of certain toads and some are prepared semi-synthetically (Table 32.1). Official digitalis is the dried leaf of *Digitalis purpurea,* the purple foxglove plant. All compounds are chemically glycosides i.e. they consist of an aglycone (genin) linked to one or more sugar moieties through an oxygen linkage. The aglycone consists of a cyclopentanoperhydrophenanthrene nucleus (steroid nucleus) to which is attached a 5 or 6 member unsaturated lactone at C-17 position. One or more hydroxyl and other substituents are present on the alycone. Sugar molecules are usually attached at C-3 (Figure 32.1).

The pharmacological activity of cardiac glycoside resides in the aglycone, and the attached sugars modify drug's solubility and cell permeability. Number of hydroxyl groups present on the aglycone determines the polarity of drug. In general, absorption, extent of protein binding and biotransformation of the individual glycosides are related directly to their lipid solubility and thus inversely to their polarity e.g. digitoxin versus ouabain. The aglycone fraction can be released from cardiac glycosides by hydrolysis.

Mechanism of Action

Cardiac glycosides bind selectively to the membrane associated Na^+-K^+-ATPase, which constitutes the Na^+-K^+ pump. Glycosides bind to the K^+ binding site of the enzyme and reversibly inhibit the pump. Inhibition of this cation pump results in progressive accumulation of Na^+ intracellularly that favours the exchange of Na^+ for Ca^{++} through Na^+-Ca^{++} exchanger (Figure 31.5, step 4). Increased accumulation of Na^+ inside the cell also slows the extrusion of Ca^{++} through Na^+-Ca^{++} exchange mechanism (Figure 31.5, step 7) by reducing the inwardly directed gradient for Na^+. This causes intracellular calcium levels to rise that in turn leads to increased release of Ca^{++} from the sarcoplasmic reticulum and hence increased contractility of the cardiac muscle. The inotropic effect of cardiac glycosides takes hours to develop

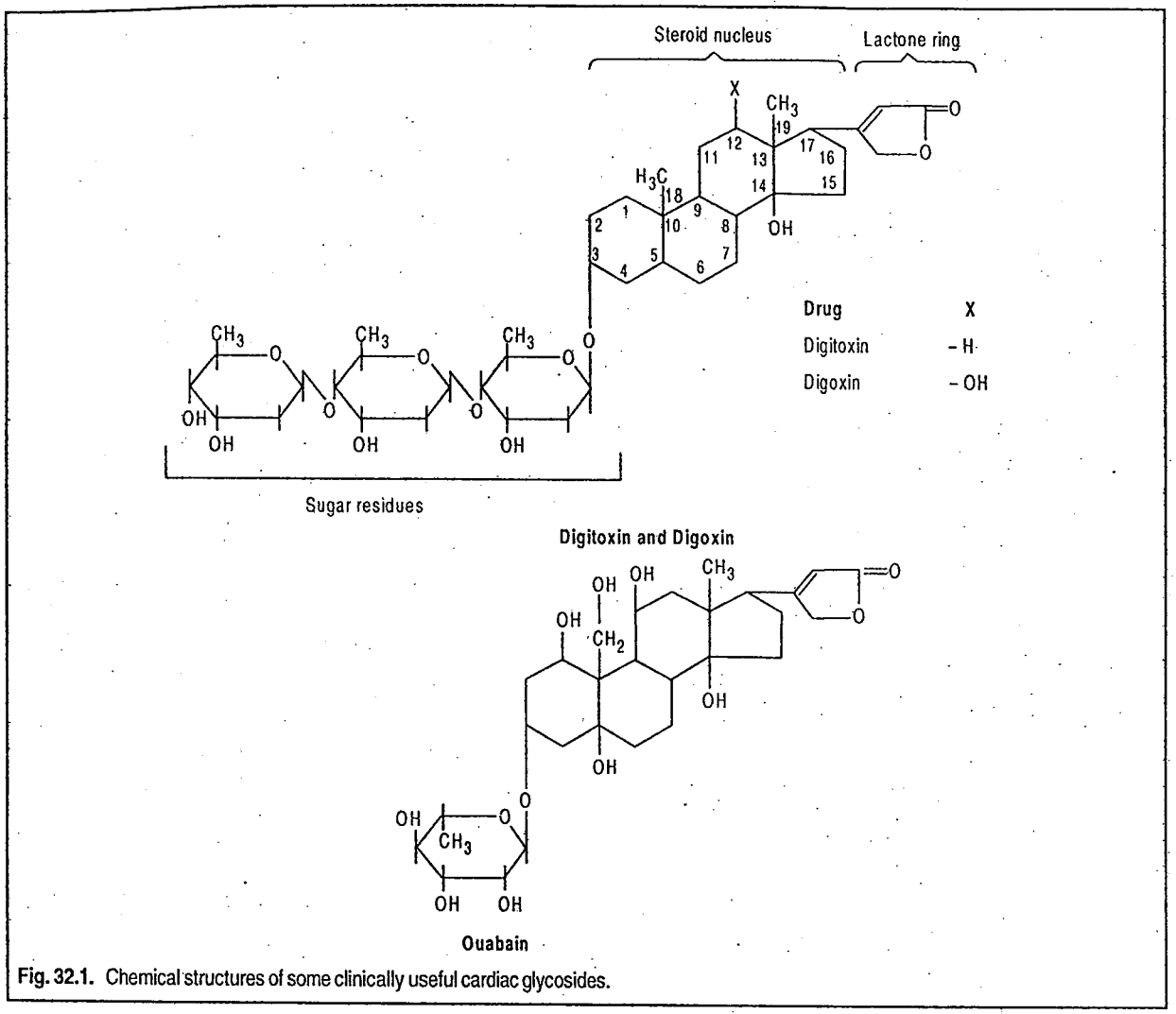


Fig. 32.1. Chemical structures of some clinically useful cardiac glycosides.

Table 32.1: Source and chemical composition of some cardiac glycosides.

Compound	Source	Aglycone	Sugar(s)
Natural			
1. Digitoxin*	*Digitalis purpurea* (leaf) *Digitalis lanata* (leaf)	Digitoxigenin	3 molecules of digitoxose
2. Digoxin*	*Digitalis lanata* (leaf)	Digoxigenein	3 molecules of digitoxose
3. Gitoxin	*Digitalis purpurea* (leaf) *Digitalis lanata* (leaf)	Gitoxigenin	3 molecules of digitoxose
4. Gitalin	*Digitalis purpurea*	Gitalligenin	2 molecules of digitoxose
5. Gitaloxin	*Digitalis purpurea*	Gitaloxigenin	3 molecules of digitoxose
6. Ouabain* (Strophanthin-G)	*Strophanthus gratus* (seed)	Ouabagenin (G-Strophanthidin)	1 molecule of rhamnose
7. Strophanthin-K	*Strophanthus kombe* (seed)	Strophanthidin	1 molecule of glucose and 1 molecule of cymarose
8. Lanatoside-C*	*Digitalis lanata*	Digoxigenin	2 molecules of digitoxose, 1 molecule of glucose and 1 molecule of acetyldigitoxose.
9. Bufotoxin	*Bufo vulgaris* (toad skin)		
Semi-Synthetic			
1. Acetyl digoxin			
2. Acetyl strophanthidin			
3. Desacetyl lanatoside*			

* = Clinically used in veterinary or human medicine.

because inactivation of Na^+-K^+-ATPase and subsequent Ca^{++} loading occurs slowly and gradually.

Inhibition of Na^+-K^+-ATPase is involved also in the toxic actions of digitalis. At high doses, excessive Ca^{++} loading of sarcoplasmic reticulum results in spontaneous cycles of Ca^{++} release and uptake producing oscillatory after-contraction and after-depolarisations. Changes in the ratio of intracellular and extracellular electrolytes can result in increased automaticity and cardiac arrhythmias.

Digoxin

Digoxin is a cardiac glycoside that occurs as bitter tasting, clear to white crystals or crystalline powder. It is derived primarily from the leaf of *Digitalis lanata*. It is practically insoluble in water, slightly soluble in alcohol and very slightly soluble in 40% propylene glycol that it used as vehicle for commercial injection. Presently, digoxin is most widely used cardiac glycoside in human and veterinary medicines and is described as the prototype drug.

Pharmacological effects

The most important effect of digoxin is produced on the cardiac muscle contractility. In addition to the cardiac effects, it affects vascular smooth muscle, kidneys, gut and central nervous system. Extracardiac effects are responsible mainly for many of the adverse effects of digoxin.

Heart: Most of the cardiac actions of digoxin are manifested in a decomposed heart than in a normal heart.

Force of contraction: Digoxin primarily acts as positive inotrope by increasing the force of myocardial contraction. The cardiac muscles contract more forcefully to the same filling causing complete ventricular emptying, improved cardiac output, reduced diastolic pressure and reduction in size of dilated heart. Duration of systole is shortened and diastole is prolonged giving greater time for ventricular filling and rest to the heart. In CHF, there is sympathetic mediated vasoconstriction, but after digoxin induced improved circulation the enhanced vasomotor tone is withdrawn that in turn reduces peripheral resistance. Digoxin increases force of contraction in normal heart also, but this is not translated into increased cardiac output because the normal heart empties nearly completely even otherwise. Further in normal heart, increased peripheral resistance (due to sympathetic vasomotor tone) and compensatory mechanisms usually counteract the positive inotropic effect of digoxin.

Heart rate: In CHF, digoxin slows the heart rate by both direct and indirect effects. In the direct action, digoxin acts on the specialised conducting tissues of heart, especially AV node, and causes an increase in refractory period and a decrease in conductivity. This cardiac slowing effect (negative chronotropy) is augmented by a more prominent vagal stimulation (indirect effect) that causes slowing of SA node rate and delayed conduction in AV node (Table 32.2). The slowing of AV conduction can progress to AV block at high concentration. The increase in vagal tone occurs by sensitisation of baroreceptors and/or stimulation of vagal centre in the brain. Improved circulation (due to positive inotropic effect) also helps to restore the diminished vagal tone and abolishes sympathetic over activity. In atria, vagal stimulation, however, produces variable effects like decrease in impulse conduction, refractory period and action potential duration, which can lead to atrial arrhythmias (Table 32.2). Prolongation of refractory period and slowing of the conduction velocity through AV node, however, protects the ventricles from atrial impulses in conditions like atrial tachyarrhythmia, atrial flutter and atrial fibrillation. The indirect vagal action manifests early and it can be blocked by atropine, whereas the extravagal action becomes prominent later and cannot be reversed by atropine.

Excitability: At low doses, digoxin enhances myocardial excitability by shifting resting membrane potential towards isoelectric level (i.e. gap between resting membrane potential and threshold potential is decreased). This occurs mainly due to enhanced intracellular Na^+ concentration that decreases negativity inside the cell. High doses, however, decreases excitability by making RMP less negative that causes fast Na^+ channels to remain in an inactivated (closed) state. This makes the cells somewhat refractory to the formation of subsequent action potential. As a result, the rate of 0 Phase depolarisation is reduced. This action is most marked in AV node and bundle of His, and may help to decrease myocardial conductivity.

Automaticity: At therapeutic doses, digoxin reduces SA and AV nodes automaticity by decreasing the slope of Phase 4 spontaneous depolarisation, which yields a reduced firing rate. This effect primarily occurs due to increased vagal tone and decreased sympathetic tone. However, the slope of Phase 4 spontaneous depolarisation is increased in Purkinje fibres resulting in enhancement of ectopic automaticity (Figure 32.2). In addition, high doses of digoxin produce coupled beats by delayed after-depolarisation (see for detail Chapter 34).

Effective refractory period: Digoxin and other cardiac glycosides influence refractory periods through direct and indirect mechanisms. Vagal dependent actions shorten the refractory period of atrial muscle fibre that tends to exacerbate atrial fibrillation rhythms. In contrast, the refractory period of AV conduction

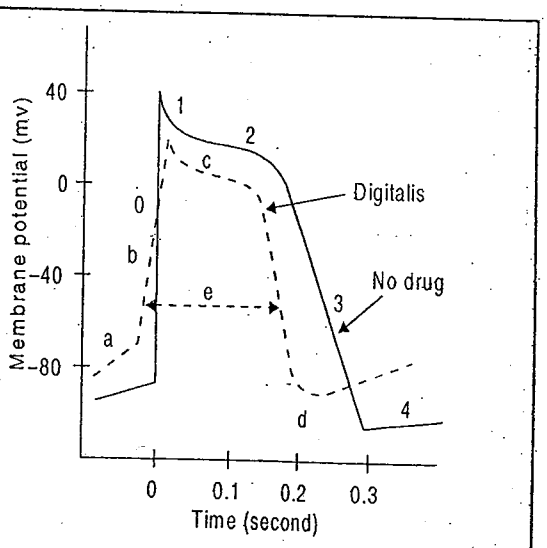


Fig. 32.2. Effect of digitalis on Purkinje fibre action potential.

(a) Digitalis causes increase is slope of phase 4 spontaneous depolarisation. This enhances automaticity.

(b) Digitalis decreases rate of depolarisation of phase 0. This favours decrease in conduction velocity.

(c) Digitalis decreases amplitude of action potential.

(d) Digitalis causes decrease (less negative) in resting membrane potential. This enhances excitability in lower dose, but decreases excitability in higher dose (see also text).

(e) Digitalis decreases refractory period and duration of action potential. This favours re-entrant arrhythmia.

system (AV node and bundle of His) is prolonged by vagal mechanisms; the maximal rate at which impulses can be transmitted is reduced. Digoxin shortens the refractory period in the ventricle that may contribute to re-entrant arrhythmia. In Purkinje fibres, there is a progressive decrease in the duration of action potential (Figure 31.2) due to faster depolarisation (refractory period shortened). The resting potential and the amplitude of action potential also decrease.

Electrocardiogram: The multiplicity of electrophysiological effects of cardiac glycosides in myocardial tissues produces equally complex changes in the ECG. The T wave becomes smaller, disappears, or may become inverted. There is depression of ST segment below the isoelectric line (due to interference with repolarisation). The P-R interval is prolonged (Figure 32.3) due to slowing of AV conduction and AV block may occur at high dose. The Q-T interval is shortened, reflecting shortening of ventricular systole. These changes are marked in diseased myocardium than normal heart.

Myocardial oxygen consumption: Myocardial oxygen consumption (MVO_2) is directly influenced by inotropic state, heart rate and wall tension. Increased contractility after cardiac glycosides causes an increase in myocardial oxygen consumption. On the other hand, reduction in heart rate and ventricular size after digitalis therapy reduces oxygen requirement of the heart. Thus, the overall effect of digitalis is improvement of ventricular performance of the failing heart without significant increase in energy requirements.

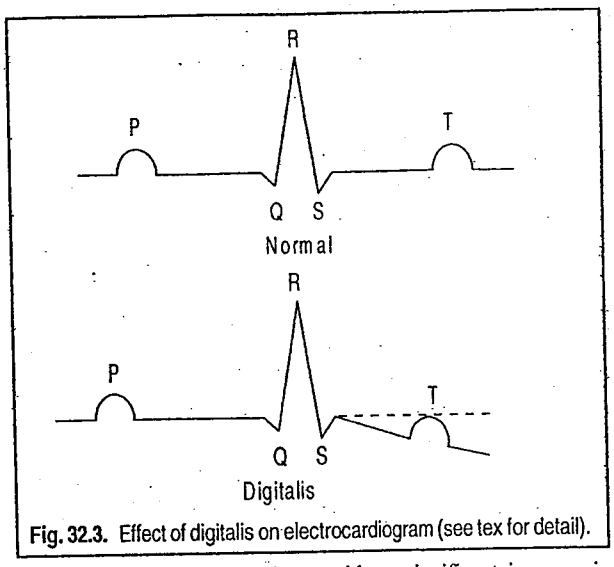


Fig. 32.3. Effect of digitalis on electrocardiogram (see tex for detail).

Extra-cardiac effects:

Blood pressure: Digitalis has no prominent effect on blood pressure. Systolic blood pressure may increase and diastolic pressure may fall in CHF patients. Systemic pressure tends to normalise and even may increase. Raised venous pressure of CHF

Table 32.2: Pharmacological effects of digoxin on the heart.

Parameter	Therapeutic dose	Toxic dose	Response/Adverse effect
1. SA node			
Automaticity	↓ (I*, D)	↓↓ (I, D*)	Decreases sinus rate.
2. Atrium			
Automaticity	↑ (I), → or ↑ (D)	→ or ↑ (D)	
Excitability	→ or ↓ (D)	↓↓** (D)	
Conduction velocity	↑ (I*), ↓ (D)	↓ (D)	Promotes atrial fibrillation.
Refractory period	↓ (I*), ↑ (D)	↑ (D)	Promotes atrial fibrillation.
Contractility	↓ (I), ↑ (D)	↓ (I)	
3. AV conduction System			
Automaticity	↑ (I), → or ↑ (D)	↑ (D)	Increases AV junctional rhythms.
Conduction velocity	↓ (I*, D)	↓↓** (I*, D)	Decreases ventricular rate in atrial fibrillation; AV block.
Refractory period	↑ (I*, D)	↑↑ (I*, D)	Decreases ventricular rate in atrial fibrillation; AV block.
4. Ventricle			
Automaticity	→	→ or ↑ (D)	
Excitability	→ or ↑ (D)	↓↓** (D)	
Conduction velocity	→ or ↑ (slightly)	→ or ↓ (D)	Extra-systole favours re-entry arrhythmias.
Refractory period		→ or ↓ (D)	
Contractility	↑↑ (D)	?	
5. Purkinje fibres			
Automaticity	↑ or → (D)	↑↑ (D)	
Excitability	↑ or → (D)	↓**	
Conduction velocity	↓	↓↓** (I, D)	Favours re-entry arrhythmias.
Refractory period	→ or ↑	↓↓**	Favours re-entry arrhythmias.

Abbreviations/symbols: D = direct effect mediated via $Na^+ - K^+$-ATPase inhibition; I = indirect action mediated via vagal stimulation (mainly) and antiadrenergic responses. Indirect effect on ventricle is negligible due to sparse vagal innervations.
↑ = increase; ↓ = decrease; → = no effect; * = predominant effect; ** = dose-dependent response.

is normalised due to removal of excess extracellular fluid and improved cardiac function.

Blood vessels: Digoxin has a mild direct vasoconstrictor action on arteriolar and venous smooth muscle. This may increase peripheral resistance in normal individuals. However in CHF, these effects are counteracted by increased cardiac output and withdrawal of sympathetic over activity; therefore, a net decrease in peripheral resistance occurs. Venous tone is improved in normal individuals as well as in CHF patients.

Kidney and diuresis: In CHF there is gradual developing inability of the heart to function normally, which is associated with decreased cardiac output. This leads to abnormal increase in blood volume and formation of oedema through diminished glomerular filtration rate and/or enhanced sympathetic mediated angiotensin and aldosterone secretions, which favour Na^+ and water retention in the kidney (Figure 32.4). After digitalisation, there is increased renal blood flow due to improved circulation and decreased sympathetic activity. This results in increased urinary output (diuresis) and relief of oedema. The retained salt and water is gradually excreted in patients with CHF. No such diuresis occurs in normal individuals or in patients with oedema due to other causes.

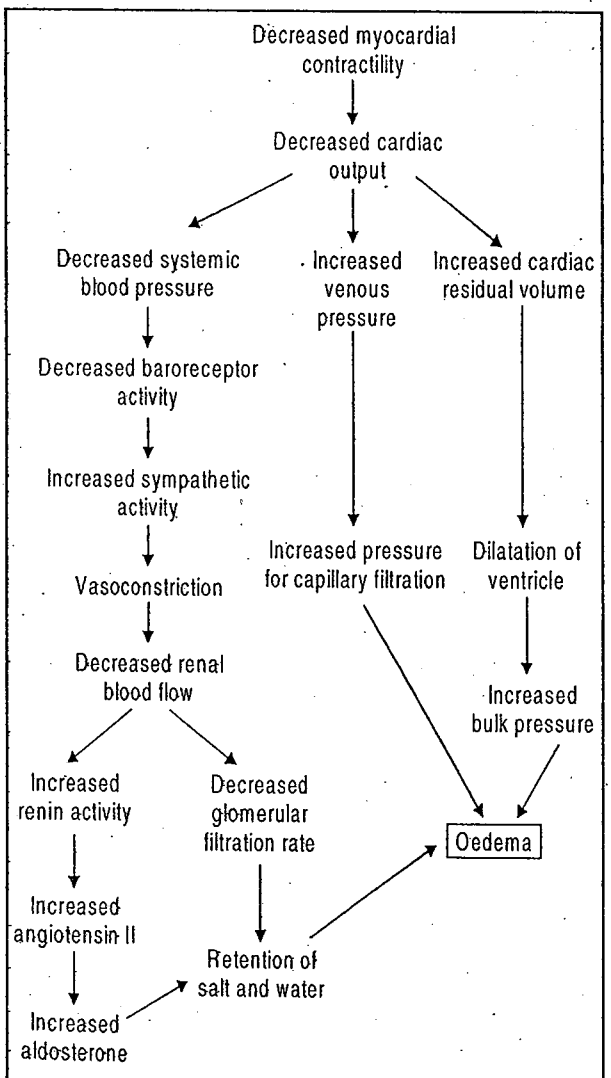


Fig. 32.4. Schematic representation of sequence of events in the formation of oedema in the congestive heart failure. Digitalis increases the myocardial contractility and causes reversal of various processes resulting in enhanced diuresis (Digitalis diuresis).

Central nervous system: Low doses of digoxin have little effect on the CNS. High doses may cause nausea and vomiting through chemoreceptor trigger zone (CTZ) activation. Still higher doses produce hyperpnoea, central sympathetic stimulation, disorientation and visual disturbances.

Pharmacokinetics

After oral administration, digoxin is absorbed mainly from the small intestine. Presence of food delays but does not alter the extent of absorption. Absorption of digoxin is also dependent on the type of oral dosage form used i.e. peak serum levels occur within 45-60 minutes after oral elixir and at about 90 minutes after oral tablet administration. At therapeutic levels, approximately 20-30% of the drug is bound to plasma proteins. Digoxin is distributed widely throughout the body with highest levels found in kidneys, heart, intestine, stomach and liver and lowest concentrations in brain and plasma. Intravenous administration results in pharmacological effects in 5-30 minutes with maximal effect in 2 hours, while oral administration results in pharmacological effects in 1-2 hours. In dogs, the therapeutic serum concentration is in the range of 1 to 2.5 ng/ml.

Digoxin is metabolised slightly (<20%) and, therefore, is excreted mainly as the unchanged drug via kidney. Digoxin undergoes enterohepatic cycling in some species. Digoxin has a plasma half-life of 24 to 55 hours in dogs, 12 to 48 hours in horses, 7 hours in cattle and sheep, and about 32 hours in cats. Due to large variation in the plasma half-life in some species, individual dosage regimen depending on the patient's response should be adopted.

Side effects/Adverse effects

All cardiac glycosides have narrow margins of safety (therapeutic index = 1.5 - 3) (Figure 32.5). Digoxin toxicosis occurs frequently, although the early systemic signs (anorexia, depression and sometimes vomiting) allow nearly all cases to be recognised before they are dangerously intoxicated. Digoxin primarily causes cardiac and extracardiac signs of toxicosis. Cardiac effects may occur before extra-cardiac signs and may include all types of arrhythmias with a resultant worsening of heart failure symptoms. More common arrhythmias include complete or incomplete heart block, bigeminy, paroxysmal ventricular or atrial tachycardia with block, and multifocal premature ventricular contractions. Because the worsening of heart disease can also cause these effects, it is essential to know effects of digoxin by monitoring its serum level or by stopping the therapy temporarily. Extra-cardiac signs of toxicity include diarrhoea, anorexia, nausea and vomiting. Neurological effects include malaise and drowsiness. Overdosage with digoxin and other cardiac glycosides may cause an excessively slow heart rate, which reduces cardiac output and causes renal dysfunction as a result of hypoperfusion.

Adverse effects of digoxin are usually related with high or toxic serum levels. Signs of toxicity are generally milder or absent when serum digoxin concentration is less than 2.5 ng/ml. Moderate signs of intoxication are associated with concentrations of 2.5 to 6 ng/ml, and severe toxicosis occurs when the levels exceed 6 ng/ml. Cats are more sensitive to digoxin, while dogs tend to be more tolerant of high serum levels. A number of factors increase the animals' susceptibility to the toxic effects of cardiac glycosides

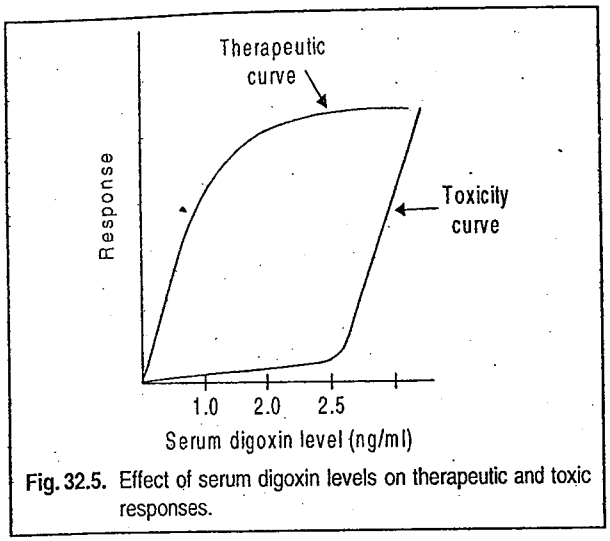


Fig. 32.5. Effect of serum digoxin levels on therapeutic and toxic responses.

including hypothyroidism, renal insufficiency, old age, obesity and hypokalaemia resulting from prolonged diuretic therapy.

Treatment: Treatment of digoxin therapy includes discontinuation of digitalis therapy and potassium depleting diuretics, if any. Use of specific antiarrhythmic agents like phenytoin (blocks AV nodal effects of digitalis), lidocaine (for ventricular arrhythmias) and propranolol are most commonly used to treat life-threatening digoxin-induced arrhythmias. Atropine may be used to treat both sinus bradycardia and second- or third-degree AV block induced by cholinergic augmentation. Digoxin immune Fab gives rapid recovery and is useful in treating life-threatening digoxin toxicity. However, it is expensive and veterinary experience with it is limited (see for detail Chapter 76).

Contraindications and precautions

Digoxin and other cardiac glycosides should not be used in conditions like ventricular fibrillation, heart block (if not due to CHF), circulatory shock, renal failure and hepatic failure. They should be used with caution in patients with severe pulmonary disease, hypoxia, incomplete AV block, acute myocardial infarction and frequent ventricular premature contractions. Animal that is hypernatraemic, hypokalaemic, hypercalcaemic, or suffering from hyper- or hypothyroid may require smaller doses. The dose of digoxin should be decreased in patients with reduced renal perfusion. Digoxin should be used with care in cats. Digoxin should not be administered IM because it causes pain and muscle necrosis. Electrolyte disorders should be corrected before digoxin or other glycosides are administered.

Drug interactions

Digoxin shows interactions with a number of drugs.

a. The concurrent administration of quinidine will increase plasma concentration of digoxin, probably by displacing the drug from tissue-binding sites. Verapamil, spironolactone and captopril may also enhance plasma digoxin concentration.

b. Antacids, cimetidine and metoclopramide may increase the amount of digoxin absorbed from the GI tract.

c. Effects of cardiac glycosides are enhanced with decrease in K^+ in plasma. This is probably related to competition between K^+ and digitalis for the Na^+-K^+-ATPase binding site i.e. increase potassium inhibits binding of cardiac glycoside to the Na^+-K^+-pump, and vice versa. Most diuretics and glucocorticoids deplete body potassium and hence enhance digitalis effect and toxicity.

d. Administration of β-adrenoceptor agonists increases the likelihood of arrhythmias by increasing ectopic automaticity.

e. Calcium synergises with digoxin and precipitates its toxicity.

f. Penicillamine may decrease serum level of digoxin.

g. Erythromycin, omeprazole and tetracyclines increase bioavailability of digoxin.

Clinical uses

In veterinary medicine, digoxin and other cardiac glycosides are used mainly for the treatment of CHF, atrial fibrillation or flutter, and supraventricular tachycardias. These drugs restore adequate circulation in animals with CHF due to poor contractile function. During supraventricular tachyarrhythmias such as atrial fibrillation or flutter, digitalis protect ventricle by slowing the ventricular rate. Cardiac glycosides also have a place in the management of CHF where it is associated with primary or secondary myocardial failure, especially in dilated cardiomyopathy. However, they are not primarily indicated for the treatment of CHF caused by valvular insufficiency or intracardiac shunts, unless there is secondary myocardial failure of tachycardia.

Administration

Digoxin is available for administration by IV or oral route. As digitalis are used to increase circulation in CHF or to slow ventricular rate in atrial fibrillation or flutter, their long-term therapy is often required to maintain therapeutic concentration. However, due to the low margin of safety, these drugs often need special dosage and administration. In dogs, when maintenance doses are given from the beginning, therapeutic plasma concentrations (1 to 2.5 ng/ml) are achieved within 3 to 5 days. Doubling the first dose often results in the therapeutic concentration being achieved more rapidly with minimal risk of toxicity. Intravenous route (rarely used now) is utilised only in emergency (e.g. atrial fibrillation) or if the patient is vomiting or is unconscious. A special practice involving initial administration of a large amount of digitalis in several divided doses (to avoid toxicity) over a relatively short period (24 to 48 hours) to quickly achieve the desired therapeutic effect is also being followed. Occasionally, this initial dose necessary to achieve the desired therapeutic response early is called the digitalisation or loading dose. This is followed by continued daily but smaller dose to maintain therapeutic concentration called the maintenance dose.

Calculation of digoxin doses should be based on lean body weight, and dosage should be reduced in obese animals and in presence of ascites. The calculated dose is usually multiplied by 0.75 for elixir and 0.85 for tablet formulation of digoxin. Alternately, dosing of digoxin on the basis of body surface area is used also for large and giant breeds of dogs. As the bioavailability of digitalis glycosides varies with the nature of dosage form and type of GI flora, the dosage preparations (also brands) should not be routinely interchanged. When changing from a tablet to an elixir formulation, the dose should be reduced by 25%. Any change in dose form during maintenance therapy may take 6 to 8 days for the effect to be evident. Elixir should be used for cats. Because digoxin and other cardiac glycosides have narrow therapeutic index, it is recommended to monitor their serum levels to help guide therapy.

Table 32.3: Pharmacokinetic properties of clinically important cardiac glycosides in dogs.

Properties	Digoxin	Digitoxin	Ouabain
1. Lipid solubility	Medium	High	Low
2. Oral absorption	Good (75-90%)	Very good (90-100%)	Poor and erratic (<10%)
3. Plasma protein binding	20-30%	70-90%	Negligible
4. Onset of action	1-2 hours (oral)	0.5-2 hours (oral)	5-10 minutes (IV)
5. Metabolism	< 20%	>80%	Negligible
6. Plasma half-life	24-55 hours	13-50 hours	–
7. Plasma concentration			
Therapeutic	1-2.5 ng/ml	15 ng/ml	–
Toxic	>2.5 ng/ml	>26 ng/ml	
8. Route of elimination (predominant)	Renal	Bile, renal	Renal
9. Route of administration	Oral, IV	Oral	IV

Dose

Dogs : 0.22 mg/m^2, PO, 2 times daily

: 0.02 – 0.06 mg/kg, PO in two divided doses on the first day (digitalisation dose) followed by 0.01 to 0.02 mg/kg, PO, in two divided doses, daily (maintenance dose).

: 0.01 – 0.02 mg/kg, rapid IV in divided doses (digitalisation) i.e. give ½ of the calculated dose IV and wait for 30-60 minutes, then give ¼ the dose and wait for another 30-60 minutes, finally give remainder, if necessary.

Cats : 0.007 – 0.01 mg/kg, PO, on alternate days.

: 0.004 mg/kg, PO, daily.

Horses : 0.02 mg/kg, PO (initial dose) followed by 0.02 mg/kg, PO, daily in two divided doses (maintenance dose).

: 0.06 – 0.08 mg/kg, PO at 8 hour intervals for 5 to 6 doses (digitalisation dose) followed by 0.01 – 0.02 mg/kg, PO (maintenance dose)

Digitoxin

Digitoxin is another cardiac glycoside obtained from the leaf of *Digitalis purpurea* and *D. lanata*, which has pharmacological properties similar to those of digoxin. Digitoxin has only one steroidal hydroxyl group (versus two for digoxin) that makes it less polar and more lipid soluble. Hence it is rapidly and nearly completely absorbed in the small intestine and highly bound to plasma proteins (dogs = 70-90%). It is also extensively metabolised in liver, partly to digoxin, and excreted in urine and faeces mainly as metabolites (Table 32.3). Unlike digoxin, dose of digitoxin is not greatly altered in renal failure, so it is more suitable in patients with renal insufficiency. In man, digitoxin has half-life of 5 to 7 days mainly due to extensive enterohepatic recirculation, whereas in dogs it is eliminated more rapidly ($t_{½}$ = 13 to 50 hours) than digoxin.

Similar to digoxin, digitoxin is indicated for heart failure or atrial fibrillation, but is used infrequently because of its unpredictable effect. Digitoxin is not routinely used in cats because of its very long half-life (>100 hours) in this species. The use of digitoxin is not recommended in horses. Because digitoxin is more protein bound and less free to combine with myocardial target sites, much larger dose is required to achieve parity with digoxin. Digitoxin exhibits similar toxic effects to the more-commonly used digoxin, including anorexia, nausea, vomiting, diarrhoea and cardiac arrhythmias. Anti-digoxin antibody fragments, the specific treatment for digoxin poisoning, are also effective in serious digitoxin toxicity.

Dose

Maintenance

Dogs : 0.02 – 0.04 mg/kg, PO, 2 to 3 times daily.

Cats : 0.005 to 0.015 mg/kg, PO, once daily.

Ouabain

Ouabain (Strophanthin-G) is a potent cardiac glycoside obtained from the ripe seeds of plant *Strophanthus gratus*. Ouabain has five hydroxyl groups on the steroid nucleus; therefore, it is absorbed poorly from the gut, does not protein bound and is excreted largely unchanged by kidneys. After IV administration, ouabain produces rapid onset (5 to 10 minutes) and short duration of action, which suits it ideally for rapid induction of emergency digitalisation. However, as emergent IV digitalisation is practised rarely now, the clinical use of ouabain has declined in veterinary and human practice.

Dose

Dogs : 0.01 mg/kg, IV, repeated hourly as necessary for effect.

: 0.022 - 0.033 mg/kg, IV, in three divided doses over 24 hours (digitalisation dose) followed by digoxin (0.011 mg/kg, PO), 2 times daily as maintenance dose.

II. PHOSPHODIESTERASE INHIBITORS

1. Methylxanthines

Methylxanthines and their derivatives such as theophylline and aminophylline are commonly used for their effects as mild stimulants and as bronchodilators. They also exert direct positive inotropic effect on heart by inhibiting the phosphodiesterase (PDE) enzyme that metabolises cAMP. Methylxanthines non-selectively block different isoforms of phosphodiesterase enzyme including the PDE III that is specific for heart and smooth muscles. The resultant increase in intracellular cAMP in cardiac muscle, in turn causes an

increase in myocardial contractility. In addition to their cardiac effects, methylxanthines have significant CNS (see Chapter 25), renal (see Chapter 39) and smooth muscle (see Chapter 46) effects. Of the methylxanthines, theophylline is the most cardiopotent. The use of methylxanthines for cardiac disease is limited to conditions those accompany respiratory disease that would benefit from bronchodilation. They are used to relieve coughing in CHF in dogs. Methylxanthines may cause tachycardia and vomiting as important side effects. The pharmacology of methylxanthines and their derivatives have been discussed in Chapter 46.

2. Bipyridine Derivatives

The cardiac bipyridines represent a relatively new class of cardioactive drugs which exert a potent inotropic effect by a mechanism different from either digitalis or sympathomimetic amines.

Amrinone

Amrinone (Inamrinone) lactate is a bipyridine cardiac inotropic agent that is structurally and pharmacologically unrelated to cardiac glycosides or catecholamines (Figure 32.6). It is mainly used in the treatment of congestive heart failure.

Mechanism of action

Amrinone is a relatively selective inhibitor of the phosphodiesterase III isoenzyme family. This isoenzyme is specific for intracellular degradation of cAMP in the heart, blood vessels and bronchial smooth muscles. Amrinone increases myocardial cAMP and transmembrane influx of Ca^{++}, thereby directly stimulating cardiac contractility. It does not inhibit Na^{+}-K^{+}-ATPase and its action is independent of tissue catecholamines and adrenergic receptors. It is also thought that amrinone produces its effects through vasodilatation, thereby reducing both pre-load and after load. This increases both the force of contraction and velocity of relaxation of cardiac muscle. Other mechanisms may also be involved in the cardio-stimulant effect of amrinone.

Pharmacological effects

Intravenous administration of amrinone causes positive inotropy and direct vasodilatation (therefore is occasionally called inodilator). Amrinone increases the rate of development of tension in myocardial fibres as well as the peak tension. Systemic and pulmonary resistance is reduced. The haemodynamic effects produced by amrinone are enhanced cardiac output, reduced ventricular filling pressure, increased ventricular ejection fraction, and improved exercise capacity. There is decrease in peripheral vascular resistance and central venous pressure, but heart rate and arterial blood pressure do not change significantly.

Pharmacokinetics

After IV administration, amrinone produces effect within 2 to 3 minutes with peak effect within 10 minutes. It exhibits low to medium protein binding (10-50%). Cardiac effects generally correlate with the amrinone serum level. In humans, amrinone is conjugated in the liver with a variable portion being excreted unchanged in urine. Heart failure may increase the elimination half-life of amrinone (also milrinone) up to two to four times than that seen in normal subjects, probably due to decrease in the hepatic blood flow in CHF patients.

Side effects/Adverse effects

Important adverse effects related to amrinone include hypotension, vomiting, diarrhoea, thrombocytopenia (particularly with long-therapy) and hepatotoxicity. Although amrinone is not inherently arrhythmogenic, in CHF patients it may increase susceptibility to development of arrhythmia. Occasionally, hypersensitivity reactions may be observed.

Treatment: There is no specific therapy to amrinone overdosage. Because it produces hypotension, circulatory support should be instituted.

Contraindications s and precautions

Amrinone should not be used when severe aortic or pulmonic valve disease is present or when the patient is hypersensitive to it. Amrinone should only be diluted with normal saline or 1/2 normal saline; no dextrose solutions should be used.

Drug interactions

Digoxin and other inotropic cardiac glycosides have an additive effect with amrinone. Disopyramide may cause excessive hypotension when used with amrinone.

Clinical uses

Amrinone is indicated mainly for the short-term IV use in severe and refractory CHF, usually as an additional drug to conventional therapy with digitalis, diuretics, etc. It is considered to be a second line agent for the management of CHF. It is presently available for IV route only. It is also effective orally, but it loses its activity after some time when given by oral route.

Dose

Dogs : 0.75 mg/kg, slow IV bolus (over 2-3 minutes) followed by constant infusion of 0.005 – 0.01 mg/kg/min.

Milrinone

Milrinone is another bipyridine compound that acts as positive inotropic and systemic arteriolar dilating agent. Similar to amrinone, milrinone selectively and potently inhibit the cAMP-specific cardiac phosphodiesterase III enzyme. Milrinone also enhances relaxation of the left ventricle by increasing Ca^{2+}-ATPase activity on the cardiac sarcoplasmic reticulum. This increases calcium ion uptake. The pharmacological properties of milrinone are related to amrinone, but it is 20 to 30 times more potent than amrinone.

Milrinone administered IV or PO results in marked positive inotropic effects with minimal chronotropic effects in animals with CHF. Onset of action following oral administration is about 30 minutes and the positive inotropic effect lasts for at least 4 hours. Milrinone is safer with a toxic to therapeutic ratio of 100 in normal dogs. Thrombocytopenia is not significant, but arrhythmias may be exacerbated in some animals. Toxicity of milrinone is primarily due to increased contractility and heart rate, particularly with high doses of milrinone. Milrinone has been associated with decreased long-term survival in chronic heart failure in man, but such fatal events are rare in dogs.

Dose

Dogs : 0.5 – 1 mg/kg, PO, 2 times daily.

3. Pyridazone Derivatives

Pimobendan

Pimobendan is a benzimidazole-pyridazinone derivative with mixed positive inotropic and vasodilatory effects. Currently it is approved for the clinical management of the signs of congestive heart failure in dogs only.

Mechanism of action

Pimobendan exerts a stimulatory myocardial effect by a dual mechanism of action. In heart it produces positive inotropic action

mainly by sensitising and increasing the binding efficiency of cardiac myofibril to the calcium ions, without causing a rise in intracellular calcium or increasing the consumption of oxygen and energy. It also inhibits function of phosphodiesterase III thereby increasing intracellular cyclic AMP concentrations. The inhibitory action on the PDE-III enzyme is more prominent on blood vessels, where it produces significant vasodilatory effects.

Pharmacological effects

Pimobendan is a potent inodilator. It stimulates the cardiac contractility with minimal effects on the heart rate. In the peripheral and coronary blood vessels, it produces significant vasodilatation. The combined inotropic and vasodilatory actions result in an increased force of contraction, decreased preload and decreased afterload. Repeated oral administration of pimobendan does not result in tachyphylaxis (decreased positive inotropic effect) or drug accumulation (increased positive inotropic effect).

Pharmacokinetics

Pimobendan is absorbed rapidly when given via the oral route and has a bioavailability of 60-65%. The oral absorption is reduced with food; therefore animals should be treated approximately one hour before feeding. After absorption, pimobendan is 90–95% bound to plasma proteins in circulation. It is oxidatively demethylated in liver to a pharmacologically active metabolite which is then conjugated with sulphate or glucuronic acid and excreted mainly via faeces. The half-life of pimobendan is 0.4 hour and the half-life of its active metabolite is 2 hours.

Side/Adverse effects

Pimobendan is generally well tolerated in dogs with vomiting and moderate chronotropic effects as side effects. Other side effects may include poor appetite, lethargy, diarrhoea, dyspnoea, weakness and ataxia. In some cases, it may produce potential arrhythmia.

Contraindications and precautions

Pimobendan use is contraindicated in patients suffering from cardiac output failure and hypertrophic cardiomyopathy. It should be used carefully in pregnant bitches as safety data in pregnant animals are not available.

Drug interactions

Pimobendan should be used cautiously with other phosphodiesterase inhibitors such as theophylline. The positive inotropic effect of pimobendan may be attenuated by the concurrent use of a β-adrenergic blocker or a calcium channel blocker.

Clinical uses

Pimobendan is used in the management of heart failure in dogs; most commonly caused by myxomatous mitral valve disease (endocardiosis) or dilated cardiomyopathy. It has shown to increase survival time and improves quality of life in patients with congestive heart failure secondary to mitral valve disease. The benefits in heart failure are caused by both positive inotropic effects and vasodilating properties. Pimobendan is also occasionally used in cats, but its therapeutic usefulness in doubtful. Pimobendan is mostly used either alone or in combination with ACE inhibitors and/or diuretics. Pimobendan is commercially supplied as 1.25 or 5 mg chewable tablets.

Dose

Dogs	:	0.2–0.6 mg/kg, PO, daily in 2 divided doses and given approximately 1 hour before feeding.
Cats	:	1.25 mg (total dose), PO, 2 times daily.

Levosimendan

Levosimendan is another inodilator with action and pharmacological effects similar to pimobendan. Levosimendan is used mainly in human medicine for inotropic support in acutely-decompensated severe congestive heart failure.

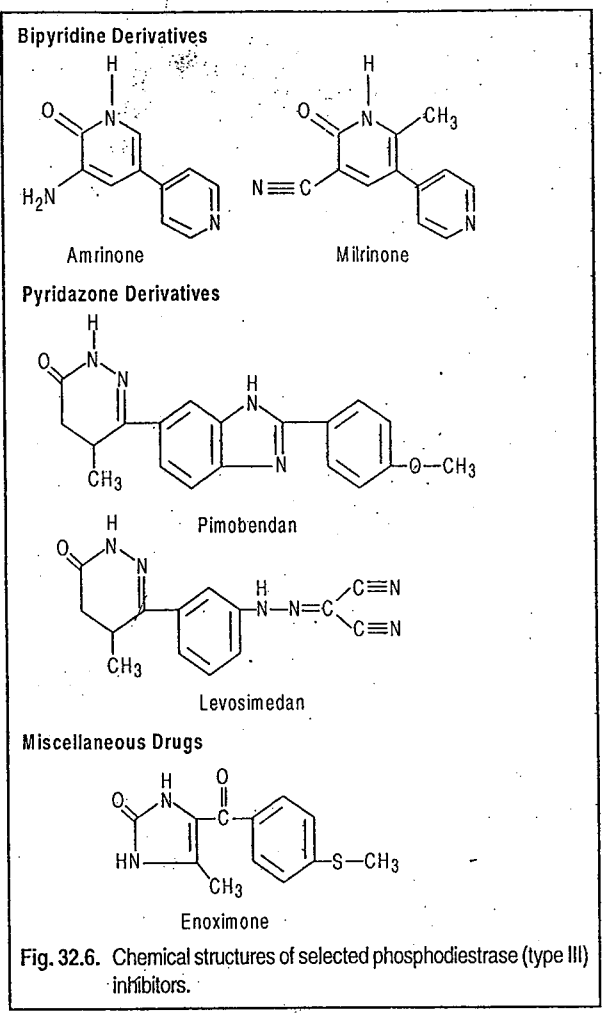


Fig. 32.6. Chemical structures of selected phosphodiestrase (type III) inhibitors.

4. Miscellaneous Drugs

Enoximone

Enoximone is an imidazole selective phosphodiesterase III inhibitor. Its principal effects are positive inotropism and vasodilatation, which are not accompanied by changes in myocardial oxygen consumption. Enoximone is metabolized mainly by oxidation to metabolite enoximone sulphoxide. Enoximone sulphoxide has the same inotropic and vasodilator activities as enoximone but is less potent and longer acting than parent drug. It is suggested that the active metabolite may contribute to some of the effects which follow enoximone administration. Enoximone is used in human medicine for short-term treatment of cardiac failure.

III. BETA-ADRENOCEPTOR AGONISTS

Sympathomimetic drugs with beta-adrenergic and dopaminergic agonistic actions have positive inotropic and vasodilator properties, which may be utilised to combat emergency pump failure. These drugs are used for cardiovascular support in the management of critically ill patients like cases under anaesthesia or those recovering from major surgery. Patients in shock, especially cardiogenic in origin, may also benefit from this type of support.

Epinephrine

Epinephrine is a non-selective adrenergic agonist that acts on both alpha- and beta-adrenoceptors. It increases both the heart rate and the contractility through $beta_1$-adrenoceptor. However, compared with other inotropic drugs, it causes greatest increase in the rate of energy usage and myocardial oxygen demand that is detrimental to the failing heart. In cardiac arrest, the drug may be used either intra-tracheally or subcutaneously via a central vein (see for detail Chapter 11).

Isoprenaline

Isoprenaline is a non-selective beta-adrenoceptor (β_1 and β_2) agonist. It increases both heart rate and contractility, but similar to epinephrine it increases myocardial oxygen demand. Isoprenaline is used occasionally in cardiac patients for short-term therapy of bradyarrhythmias or AV block. Its routine use in heart patients is restricted by its potential to produce tachycardia and some other arrhythmias. The pharmacology of isoprenaline has been discussed in Chapter 11.

Dobutamine

Dobutamine is a synthetic drug that selectively stimulates $beta_1$-adrenoceptor in heart. It stimulates cardiac contractility with minimal effect on heart rate or systemic vascular resistance. It is the preferred drug for short-term therapy of refractory congestive heart failure. The pharmacology of dobutamine has been discussed in Chapter 11.

Dopamine

Dopamine is a mixed acting catecholamine with both direct and indirect actions. Dopamine is most commonly available as a solution that is administered by constant IV infusion in cardiogenic or endotoxic shock and oliguria. Cardiac arrhythmias may occur due to β-adrenergic activity. The pharmacology of dopamine has been given in Chapter 11.

IV. MISCELLANEOUS AGENTS

Calcium

Calcium salts (gluconate and chloride) are used occasionally as cardiac stimulants by a slow injection or infusion. Calcium must be administered carefully because it can cause cardiac rigor and standstill at high doses. Calcium gluconate should be preferred to calcium chloride.

Glucagon

Glucagon, a polypeptide hormone produced by the alpha cells of the islets of Langerhans, has a potent positive inotropic activity in addition to its hyperglycaemic action (see for detail Chapter 53). The use of glucagon in refractory CHF is under investigation.

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CHAPTER 33 ANTIARRHYTHMIC DRUGS

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Antiarrhythmic (Anti-dysrhythmic) drugs are a group of agents used to prevent or treat irregularities of cardiac rhythm. In veterinary medicine, antiarrhythmic drugs are considered only when arrhythmia is dangerous to life or is affecting normal activities of animal. Although antiarrhythmic drugs may successfully control abnormal heart rhythms, minimisation or exclusion of precipitating factors is very important in the therapy of cardiac arrhythmias. If the primary disease is treated, the arrhythmia may also resolve, even in absence of cardiac therapy. Therefore whenever rhythm disturbances are detected, the animal should be thoroughly examined for presence of primary myocardial disease and various precipitating factors, and decision on use of antiarrhythmic drug taken.

CARDIAC ARRHYTHMIA

Cardiac arrhythmia (Dysrhythmia) is a broad term used for a large number of conditions which produce an abnormality in the rate, regularity, origin, or conduction of cardiac impulses such that the normal function of heart is disrupted with loss of atrioventricular synchrony. Cardiac arrhythmia often results in alterations in heart rate, regularity of heartbeats, filling of ventricles by auricles, time relationship of atrial and ventricular contraction, or myocardial contractility. The factors those precipitate cardiac rhythm disturbances include myocardial diseases (e.g. congestive heart failure and myocarditis), CNS diseases, renal failure, adrenal or thyroid diseases, hypoxia, ischemia and infarction of cardiac muscle, endocrine and metabolic disturbances, changes in serum pH and electrolyte concentration, excessive stretching of cardiac tissue, excessive sensitivity to autonomic regulators, mechanical trauma, drugs and cardiotoxic substances.

ARRHYTHMOGENESIS

Cardiac arrhythmias are generated mainly by alterations in either impulse generation (abnormal automaticity) or impulse conduction (abnormal conductivity), or combination of both.

I. Alterations in Impulse Generation (Abnormal Automaticity)

Automaticity refers to the characteristics of certain cardiac pacemaker tissues to spontaneously depolarise during phase 4 of their action potential and generate cardiac impulse. As discussed earlier in Chapter 31, the SA node initiates the origin of cardiac impulse and acts as a normal pacemaker. This normal impulse originating from SA node initially causes both atria to contract, activates atrioventricular (AV) node and then spreads through both ventricles via Bundle of His and Purkinje fibres causing a synchronised contraction of the heart muscle. However under some abnormal conditions, alterations of normal impulse

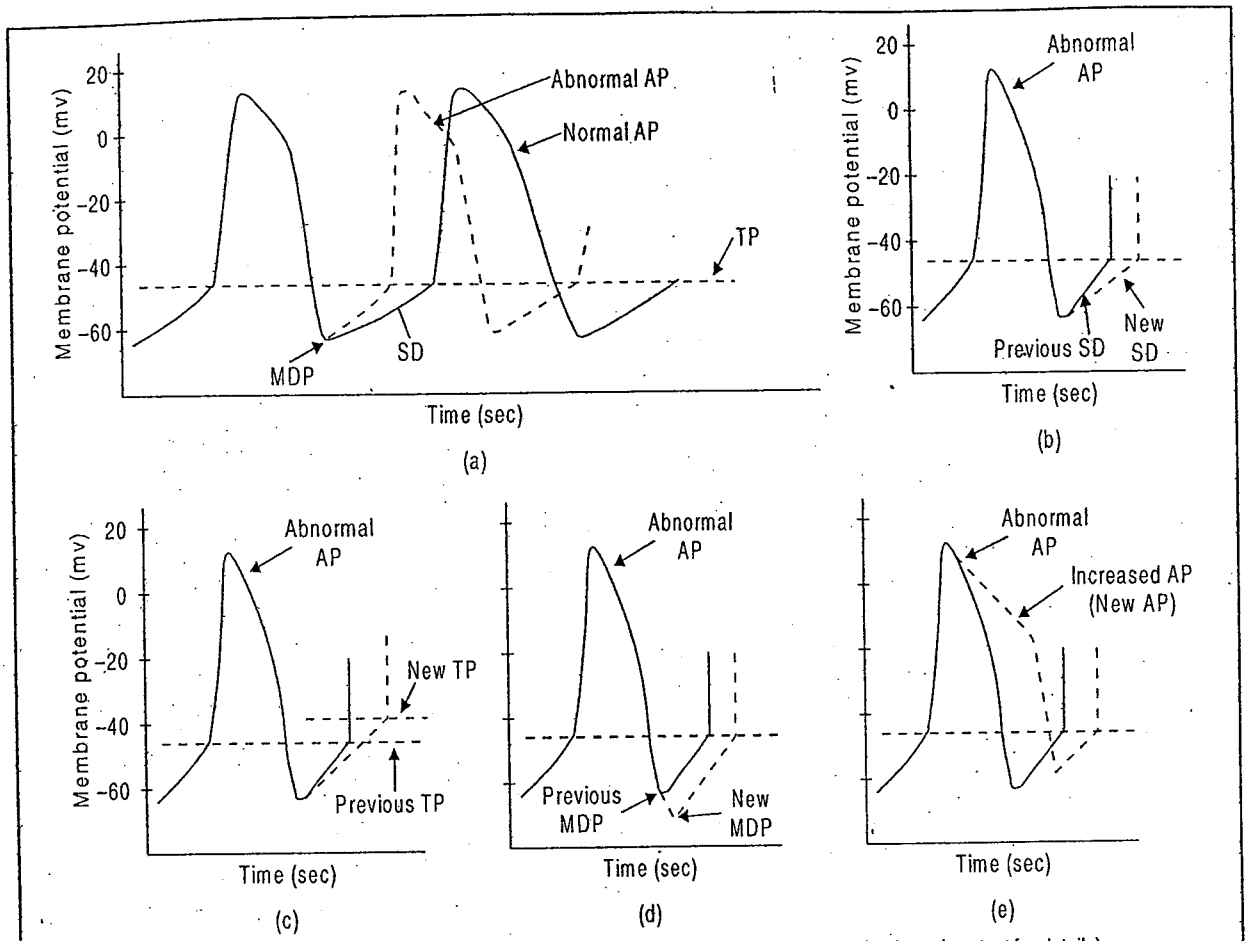


Fig. 33.1. Schematic representation of enhanced automaticity in pacemaker cells and its correction by drugs (see text for details).
(a) Increase in slope of SD causes ehanced automaticity. Normal AP of pacemaker cell is also shown for comparison.
(b) Decrease in slope of SD (new SD) by drugs restore normal AP and suppresses enhanced automaticity.
(c) Increase in TP to less negative value (new TP) by drugs suppresses enhanced automaticity.
(d) Increase in MDP to more negative value (new MDP) by drugs suppresses enhanced automaticity.
(e) Increase in duration of action potential may slow automatic rhythms.
Abbreviations: AP = action potential; MDP = maximal diastolic potential (resting membrane potential); SD = spontaneous depolarisation; TP = threshold potential.

generation occur due to either change in the automaticity of normal pacemaker (SA node) cells or development of automaticity in latent pacemaker/non-automatic tissues. When impulses propagate from a region of altered normal or enhanced abnormal automaticity to excite rest of heart, the arrhythmia may ensue.

1. Abnormal automaticity of SA node

The sinoatrial node normally exhibits the fastest rate of phase 4 spontaneous depolarisation and hence has a higher automaticity than the rest of the heart and, therefore, is usually responsible for initiating each heart beat. Any abnormal increase in phase 4 depolarisation may result in enhanced automaticity in SA node. This results in generation of more heart beats, a condition called sinus tachycardia. When the SA node automaticity is suppressed, it results in lesser number of heart beats and in sinus bradycardia. Complete failure of SA node to form action potential may result in sinus arrest. Conditions those increase SA automaticity and produce sinus tachycardia may include sympathetic nervous system over activation, and those suppress automaticity and produce sinus bradycardia include damage to the node. Sinus tachycardia or bradycardia also occur physiologically (e.g. during exercise or stress) and may not necessarily lead to arrhythmia.

2. Enhanced automaticity in latent pacemaker/non-automatic tissues

Any abnormal suppression of automaticity in SA node or increase in the rate of depolarisation during phase 4 at sites other than SA node can cause normally quiescent parts of the heart to take on a spontaneous rhythm. In addition to latent/ auxiliary pacemaker tissues (e.g. AV node and His-Purkinje system), the abnormal automaticity can occur even in non-automatic tissues such as atrial, ventricular or Purkinje fibres. Such a site of the heart that initiates a latent impulse is called an ectopic focus and the heartbeat is called ectopic beat. The latent pacemaker may cause a single premature beat now and then, or if the ectopic focus fires more often than the sinoatrial node, can produce a sustained abnormal rhythm. Such ectopic beats may arise even when SA node is the dominant pacemaker causing sinus irregularities or, more often, in patients with abnormal sinus bradycardia/sinus arrest. The generation of impulse from ectopic foci often leads to many types of arrthymias.

Enhanced automaticity in latent pacemakers is thought to involve the deviation of potassium ion currents in addition to reactivation of both sodium and calcium ion channels which

carry the depolarising current to initiate ectopic action potential. Arrhythmias due to increased automaticity in subsidiary or latent pacemakers result from myocardial ischemia or damage, hypokalaemia, or digitalis intoxication. Beta-adrenergic stimulation, circulating catecholamines, acidosis and mechanical stretch of cardiac muscle increase phase 4 slope and so accelerate pacemaker rate. More negative threshold potential due to ischemia or less negative resting membrane potential increases also automaticity. On the other hand, drugs like acetylcholine/ vagal stimulation reduces pacemaker rate both by decreasing phase 4 slope and by hyperpolarisation (making the diastolic membrane/resting membrane potential more negative).

Correction of altered automaticity

a. ***Suppressed automaticity of SA node:*** The suppressed automaticity of normal pacemaker cells (SA node) can be enhanced by blocking parasympathetic action on the heart (e.g. atropine). This helps to restore pacemaker rate of SA node by elevating the suppressed slope of phase 4 depolarisation to normal levels. Another possible approach is to elevate the slope of spontaneous depolarisation by administration of β-adrenoceptor agonists.

b. ***Enhanced automaticity:*** Many antiarrhythmic drugs suppress the enhanced automaticity in cardiac automatic/ non-automatic fibres by decreasing the frequency of discharge. The effect of such drugs is more pronounced in cells with ectopic pacemaker activity than in normal cells. These drugs act by one or more of the following modes as depicted in Figure 33.1:

i. *By decreasing slope of phase 4 spontaneous depolarisation:* Some drugs (e.g. quinidine, lidocaine, procainamide and β-adrenoceptor blockers) act by decreasing the rate of spontaneous depolarisation. This decreases the ectopic foci and restores normal sinus rhythm without affecting maximal diastolic potential or threshold potential.

ii. *By increasing threshold potential to less negative value:* Some drugs (e.g. quinidine, procainamide and propranolol) act by increasing the threshold potential to less negative value. This increases the time required to reach threshold potential, which in turn decreases automaticity. Blockade of Na^+ or Ca^{++} channels usually results in altered threshold.

iii. *By increasing maximal diastolic potential to more negative value*: By increasing the maximal diastolic potential to more negative value, some antiarrhythmic drugs (e.g. adenosine and acetylcholine) increase the time before threshold potential is reached. This helps to decrease the enhanced automaticity.

iv. *By increasing duration of action potential:* Increasing the duration of action potential decreases also the number of beats generated by enhanced automaticity. Blockade of cardiac K^+ channels prolongs the action potential.

3. After-depolarisations and triggered automaticity

Under certain pathophysiological conditions, a normal cardiac action potential may be interrupted or followed by an abnormal depolarisation (single or multiple depolarisations). This secondary depolarisation generated during repolarisation (pre-mature action potential) or immediately after the action potential is called after-depolarisation. If the after-depolarisation reaches threshold level, it may give rise to secondary upstrokes which can propagate and create abnormal rhythms. As these abnormal secondary upstrokes occur only after an initial normal or triggering upstroke, they are generally called triggered rhythms and the abnormal automaticity is termed triggered automaticity. Two major forms of after-depolarisations have been identified - early after-depolarisation and delayed after-depolarisation.

a. ***Early after-depolarisation (EAD):*** Early after-depolarisations occur prior to complete repolarisation and are associated with prolongation of the cardiac action potential. In this type, the repolarisation during phase 3 is interrupted by a second depolarization and the membrane potential oscillates. If the amplitude of oscillations is sufficiently large, a triggered beat can arise from the EAD (Figure 33.2). The EAD-mediated triggering *in vitro* and clinical arrhythmias are most commonly seen when the underlying heart rate is slow, extracellular K^+ is low, and certain drugs (antiarrhythmics and others) which prolong action potential duration are present. When an EAD is present, sympathetic stimulation can increase the likelihood of triggered beats. Sometimes, EADs may cause a specific ventricular arrhythmia termed as *torsades de pointes* (twisting of points), which is characterised by QRS complexes which appear to 'twist about the isoelectric line' and may progress to ventricular fibrillation. The EADs usually disappear with increasing heart rate.

The cellular mechanism responsible for EADs is unknown. As EADs usually occur during terminal portion of the plateau phase (phase 2) or early portion of repolarisation (phase 3), they are believed to be dependent on the inward movement of calcium ion for their initiation. When there is a prolongation of the action potential duration, the opportunity for the calcium current to flow is also more. Factors those predispose to EADs include bradycardia, low extracellular K^+ and certain drugs, including some antiarrhythmics. Bradycardia predisposes to EADs by prolonging the action potential duration and hypokalaemia by interfering with the repolarising potassium currents and prolonging repolarisation. The combination of diuretic-induced hypokalaemia with antiarrhythmic drugs which prolongs repolarisation (e.g. class I agents quinidine and disopyramide, and class III agents amiodarone and sotalol) may be extremely arrhythmogenic.

b. ***Delayed after-depolarisations (DAD):*** Delayed after-depolarisations occur immediately after completion of the action potential i.e. after full repolarisation. In this type, after the resting membrane potential (RMP) is attained, there is an abnormal oscillation of phase 4 of the action potential. If this after-depolarisation reaches threshold, a triggered beat or beats may occur (Figure 33.2). The DADs have been associated with abnormal intracellular load and are carried by sodium ions. Factors which predispose to delayed after-depolarisations include excessive adrenergic activity, digitalis toxicity and high intracellular Ca^{2+}. Increased sympathetic

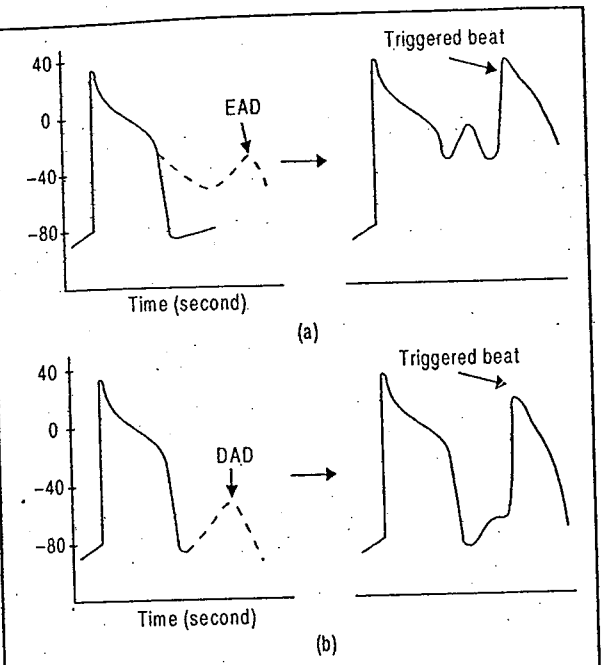


Fig. 33.2. Schematic representation of after depolarisations and triggered automaticity in a non-automatic myocardial fibre.
(a) Early after-depolarisation leading to triggered beat.
(b) Delayed after-depolarisation leading to triggered beat.
Abbreviations: EAD = early after-depolarisation; DAD = delayed after-depolarisation.

activity may increase the amplitude of DADs, which then reach threshold levels and initiate an action potential. Increased sympathetic activity or circumstances those cause cardiac myocyte calcium overload (e.g. digitalis toxicity) induce DADs mainly in ventricular or Purkinje cells. Unlike EADs, tachycardia and rapid pacing exaggerate DADs, probably by the rapid repetitive opening of calcium channels.

Correction of after-depolarisations and triggered automaticity

Antiarrhythmic drugs may block arrhythmias due to after-depolarisations either by interference with the inward current (usually Na^+ or Ca^{++} channels) that is responsible for the upstrokes, or by inhibition of development of after-depolarisation. Calcium channel blockers and low external calcium can inhibit the production of DADs. Verapamil blocks development of DADs (oscillatory current) and quinidine blocks Na^+ channels (elevates threshold required to produce abnormal stroke). Both these drugs are useful in treating arrhythmias due to digitalis induced DADs. Lidocaine, a class I-B antiarrhythmic drug and inhibitor of sodium channel, also inhibits oscillatory current, probably by a secondary reduction of internal calcium as the internal sodium falls.

The EADs can be inhibited by shortening of action potential duration. Mg^{++} can inhibit triggered beats arising from EADs without normalising repolarisation. Preventive treatment of *torsades de pointes* includes β-adrenoceptor blockade.

II. Alterations in Impulse Conduction

Alterations in impulse conduction may produce arrhythmia through mechanisms of re-entry and conduction block.

1. Re-entry

Re-entry (variously named re-entrant excitation, re-entrant tachycardia, reciprocating tachycardia, circus movement, and reciprocal or echo beats) is a phenomenon in which an impulse re-circulates in the heart and causes repetitive activation without need for any new impulse to be generated. These circuits may be fixed or stationary or may move within the myocardial substrate (as occurs with spiral waves). Re-entry phenomenon is a very common mechanism of arrhythmias, especially in cases of myocardial ischemia, inflammation or injury. It is caused by an imbalance of conduction velocity and refractoriness in cardiac tissues. It requires the presence of a myocardial tissue(s) with specific electrophysiological properties of slowed conduction and unidirectional block. The re-entry may occur in any region (functionally defined re-entry) or may follow a specific anatomical pathway (anatomically defined re-entry).

a. ***Functionally defined re-entry:*** Functionally defined re-entry is not restricted to any anatomical distinct region or pathway but may occur in any region of myocardium. Normally when a depolarisation wave reaches a refractory area in the heart, it travels to both sides around the area. Thus a single impulse becomes two, and two becomes four (Figure 33.3). In this way, many new wave fronts are continually being formed in the heart by a progressive chain reaction until there are many small depolarisation waves travelling in many directions at the same time.

Functional re-entry is usually seen when a region/ring of tissue develops the twin properties of slow conduction of action potential and an ability to conduct action potential in only one direction (unidirectional block). Each involved pathway of the circuit must also be capable of conducting an impulse in an antegrade and retrograde direction. For example, consider a single Purkinje fibre with two conduction pathways to ventricular muscle. An impulse normally travels down both limbs (A and B in Figure 33.4l) of the conduction pathway and die out within the ventricle when two impulses meet. These impulses die out because impulse of each limb meets a refractory tissue that has just been excited by the other limb. In case of myocardial ischemia or injury in one of the pathways (say A in Figure 33.4), the impulse may only be conducted in one limb (B) because there is an area of unidirectional block in other damaged limb (A). This area of unidirectional block in limb A prevents depolarisation (conduction) in the antegrade (forward) direction, but allows passage of

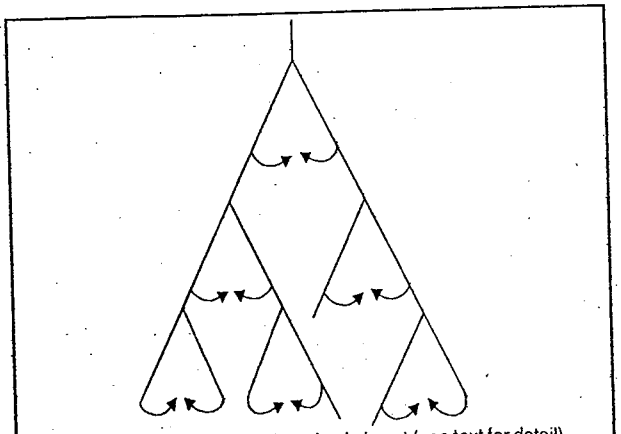


Fig. 33.3. Spread of cardiac impulse in heart (see text for detail).

impulse in retrograde (backward) direction. If the cardiac impulse is propagating in a normal fast speed, it may return to the damaged limb (A) and cross the unidirectional block in retrograde fashion and get extinguished by the refractory cells. This will not allow re-entry into the circle (Figure 33.4, II). In case the myocardial pathology has produced unidirectional block in damaged area as well as slow conduction of impulse, then the impulse (travelling at slow speed) will cross the damaged area in retrograde fashion and encounter the cells after their refractoriness period (cell are ready to be re-excited). It will re-excite the cells and then re-enter the conduction pathway from the point of bifurcation and initiate a second beat (Figure 33.4, III). This short-circuit pathway re-entry results in re-excitation of ventricular muscle causing a pre-mature ventricular beat (single re-entry) or sustained ventricular arrhythmia (multiple re-entry). The movement of an action potential around and around a non-conducting centre is called the circus movement. Re-entry circuits are responsible for atrial flutter, most paroxysmal supraventricular tachycardia, and dangerous ventricular tachycardia.

For re-entry to occur, the wavelength (effective refractory period x conduction velocity) of the re-entrant impulse must be shorter than the length of the circuit, so that the tissue into which the impulse is re-entering has time to recover excitability. Therefore in addition to slow conduction (as above), shortening of refractory period or prolongation of conduction pathway may permit also re-entry phenomenon. Slow conduction in re-entrant circuit is associated with depression or inhibition of fast sodium channels with residual slow calcium channel activity, resulting mainly from injury (blockage) to the conduction system, ischemia of muscle, high blood potassium levels, or many other factors. A shortened refractory period commonly occurs in response to various drugs such as epinephrine or after repetitive electrical stimulation. A long pathway typically occurs in dilated hearts.

b. ***Anatomically defined re-entry:*** This type of re-entry occurs in anatomically defined circuits i.e. specific pathways. For example, atrioventricular re-entrant tachycardia occurs in Wolff-Parkinson-White (WPW) syndrome. Normally, the atrial muscle is separated from the ventricular muscle by a continuous fibrous barrier that prevents passage of cardiac impulses between the atria and ventricles through any other route besides forward conduction through the AV bundle. However

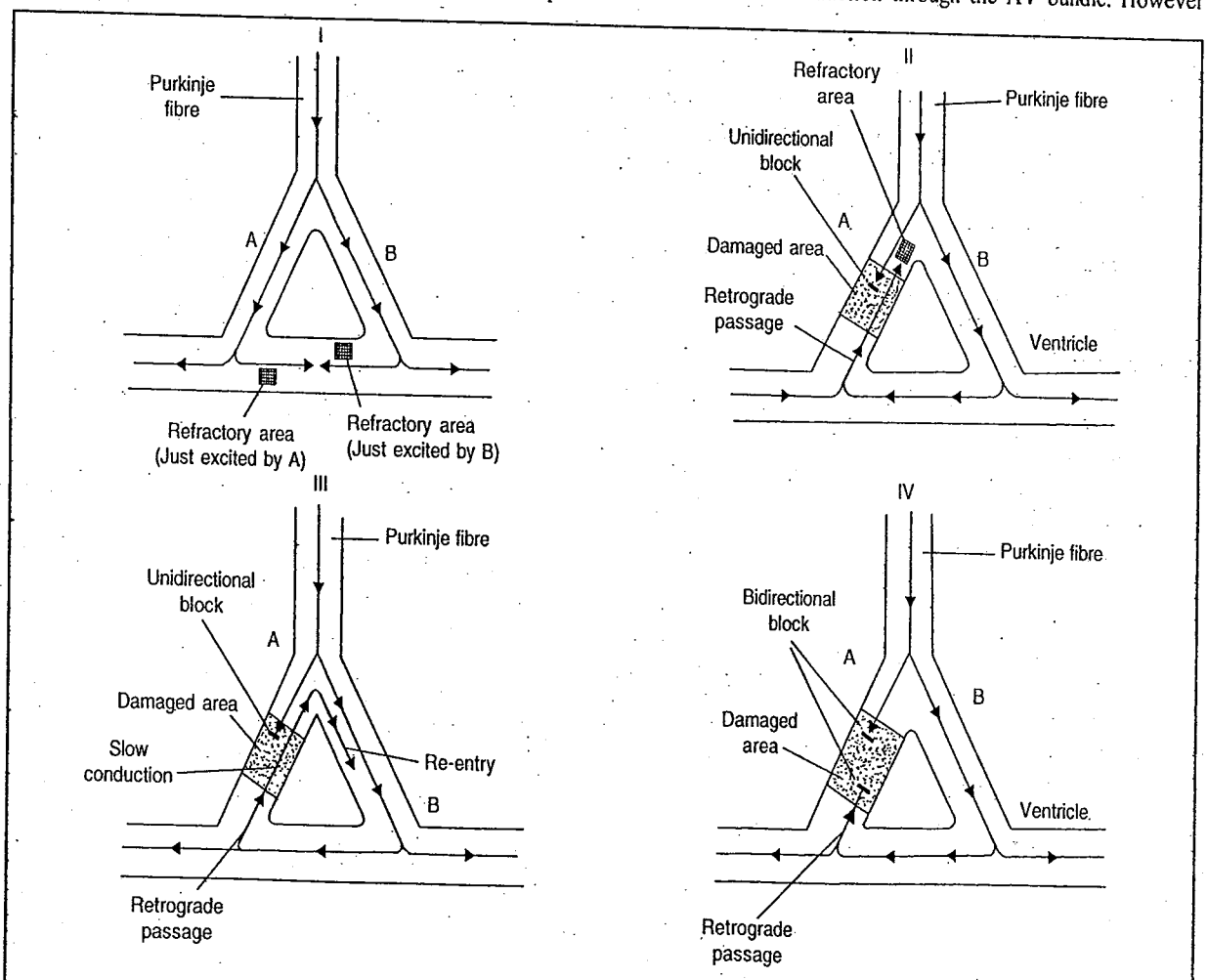


Fig. 33.4. Schematic representation of functionally-defined re-entry circuit at the Purkinje fibre-ventriular junction (see text for detail).
I : Normal conduction
II : Normal conduction and unidirectional block.
III : Slow conduction and unidirectional block.
IV : Bidirectional block produced by drug effect.

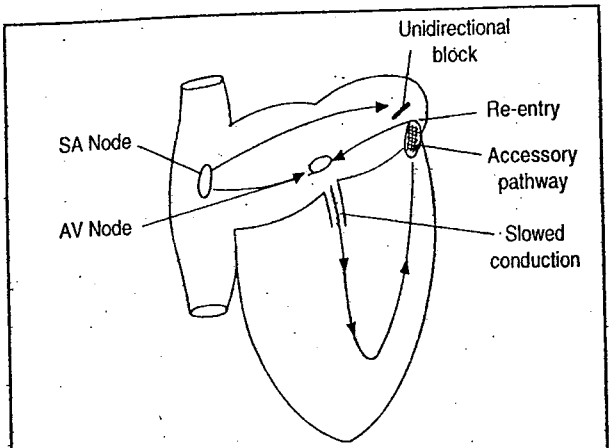


Fig. 33.5. Schematic representation of anatomically defined atrioventricular re-entry circuit through accessory pathway (see text for detail).

under certain abnormal conditions (e.g. WPW syndrome), an accessory atrioventricular muscular pathway develops which allows retrograde passage of impulse from ventricle to atrium. In case the cardiac impulse is propagating at normal speed, it does not re-enter atrium because it finds atrial cells in refractory state. However, a slow conducting impulse finds the atrial cells in an advanced state of recovery and re-excites it, setting up the re-entrant/circus movement (Figure 33.5). In such a case, passage of impulse from atrium to ventricles is not allowed due to the unidirectional block at the accessory pathway. A premature impulse may be conducted more slowly and is more likely to produce circus movement.

Similar anatomically defined re-entry may occur in the region of AV node (AV nodal re-entrant tachycardia) and in atrium (atrial flutter).

Correction of re-entry

Functionally defined re-entry: Antiarrhythmic drugs may prevent functionally defined re-entry by one or more of the following mechanisms.

(*i*) By converting a unidirectional block to a bi-directional block (Figure 33.4, IV). This will not allow retrograde passage of impulse through damaged area e.g. quinidine and procainamide.

(*ii*) By eliminating unidirectional block and normalising bi-directional conduction. This will allow antegrade passage of impulse through damaged area e.g. lidocaine and phenytoin.

(*iii*) By prolonging the duration of effective refractory period. In fast response tissues, refractoriness can be prolonged by delaying the recovery of Na^+ channel from inactivation or blocking of Na^+ channels (e.g. quinidine). In slow response tissues, Ca^{++} channel block prolongs refractoriness.

(*iv*) By acceleration of conduction in an area of slow conduction. Lidocaine may exert such an effect under some experimental conditions.

Anatomically defined re-entry: Anatomically defined re-entry may be terminated by drugs which slow propagation of the action potential. In the WPW-related arrhythmia, propagation of AV nodal refractoriness and slowing of AV nodal conduction such as Ca^{++} channel blockers, β-adrenoceptor antagonists or digitalis glycosides are likely to be effective.

2. Conduction Block/Heart Block

Under certain conditions, the conduction of impulse through nodal tissues is either decreased or blocked. If this happens to the AV node, there may be partial to complete heart block. Three degrees of severity of AV node block are characterised.

a. ***First degree AV block:*** It is characterised by a delay of conduction from the atria to the ventricles, but no actual blockade of conduction. It results in prolonged P-R interval.

b. ***Second degree AV block:*** It occurs when the AV block is partial i.e. some sinus beats are not transmitted to the ventricle. This may appear in a regular pattern (e.g. 2: 1 or 3: 1) in which every second or third impulse is transmitted. In such cases, the ventricular rate and conduction output will be low.

c. ***Third degree AV block:*** It occurs when the AV conduction fails completely i.e. no impulse is propagated from the atria into the ventricle.

Atrioventricular (AV) node block can be caused by toxins, viral or bacterial infections, ischemia, congenital heart defect, cardiac fibrosis and damage to AV node. Parasympathetic activity often increases the refractory period of AV node cells and can create or exaggerate AV node block.

Correction of conduction block

Drugs which block parasympathetic actions on the heart (e.g. atropine) might reduce the AV node refractory period and help to overcome a blocked state. Use of sympathomimetics (e.g. isoprenaline) may also help to overcome this problem by activating β_1-adrenoceptors in the heart. If drug treatment fails to correct AV node block, then the artificial pacemaker needs to be applied to the ventricles.

Types of Arrhythmia

Arrhythmias are generally classified according to the heart rate (increased or decreased) and site of origin of the abnormality.

1. Tachyarrhythmias

Tachyarrhythmias are abnormalities in cardiac rhythm in which the atrial or ventricular rates, or both are abnormally high. Cardiac tachyarrhythmias result from abnormal action potential formation (ectopic pacemaker) or from abnormal action potential conduction (re-entry). Depending on the site of origin, severity and nature of tachycardia, following important types of tachyarrhythmias have been identified:

a. ***Atrial tachycardia:*** It is a type of tachycardia that originates from an ectopic pacemaker within the atria.

b. ***Sinus tachycardia:*** It originates from the SA node pacemaker cells and is characterised by rapid sinus rhythms (dogs >180 beats/minute and cats >220 beats/minute).

c. ***Junctional tachycardia:*** It originates from ectopic pacemaker within the AV node or first part of the AV bundle. It is also called AV nodal tachycardia.

d. ***Supraventricular tachycardia:*** It is a general term used for any tachycardiac rhythm originating above the

ventricular tissue. It normally encompasses sinus tachycardia, atrial tachycardia and junctional tachycardia.

*e. **Ventricular tachycardia:*** It originates from an ectopic pacemaker within the ventricles. In this type, ventricles do not relax long enough between contractions for adequate filling.

*f. **Atrial flutter:*** It is an extremely rapid but coordinated atrial tachycardia. In atrial flutter, electrical signal travels as a single large wave front always in one direction (Figure 33.6). Atrial flutter does not lead to ventricular activities because of large refractory period of the AV node.

*g. **Atrial fibrillation:*** It is an extremely rapid and uncoordinated atrial tachycardia. In atrial fibrillation, atrial contractions are so intense that they lose synchrony i.e. action potential is propagated in random way throughout the atria and no blood is pumped. Atrial fibrillation usually does not lead to ventricular fibrillation due to long refractory period of AV node cells.

*h. **Ventricular fibrillation:*** It is an uncoordinated ventricular tachycardia. In ventricular fibrillation, the action potential spreads randomly and continuously throughout ventricles; thus, each tiny region of the ventricle wall contracts and relaxes at random. It mostly leads to death.

*i. **Paroxysmal tachycardia:*** It occurs due to abnormalities in any portion of the heart, which cause rapid rhythmical discharges of impulses spreading in all directions throughout the heart. It is frequently caused by re-entrant pathways which set up local repeated self-excitation.

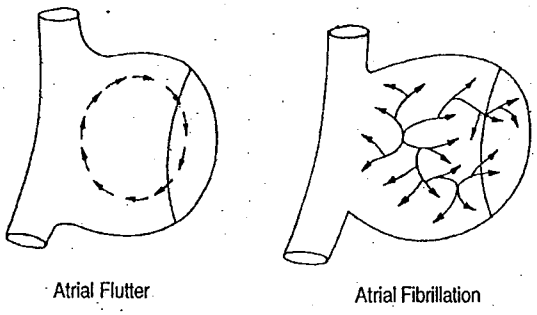


Fig. 33.6. Schematic representation of development of atrial flutter and atrial fibrillation.

2. Bradyarrhythmias

Bradyarrhythmias are abnormalities in cardiac rhythm in which the heart rate is abnormally low. Similar to tachyarrhythmias, the bradyarrhythmias result from abnormal impulse generation and/or abnormal conductivity. Bradyarrhythmias are less important than tachyarrhythmias. Following terms are often used to describe various types of bradyarrhythmia.

*a. **Sinus arrest:*** It is the complete failure of SA node to form action potential.

*b. **Sick sinus:*** It is less extreme form of sinus arrest. It is characterised by sluggish depolarisation of the SA-node pacemaker cells.

*c. **AV-block:*** It is the partial (slowed) or complete blockade of impulse conduction through AV node.

*d. **Cardiac arrest:*** It results from cessation of all rhythmical impulses of the heart. It mainly occurs during deep anaesthesia when severe hypoxia prevents muscle fibres and conductive fibres from maintaining normal electrolyte concentration differentiation across their membranes. This affects excitability in such a way that the automatic rhythmicity disappears.

ANTIARRHYTHMIC DRUGS

Antiarrhythmic (Antidysrhythmic) drugs are a group of pharmaceuticals used to suppress abnormal rhythms of the heart, even those caused by myocardial diseases. The antiarrhythmic drugs are considered in therapeutics only when arrhythmia is life-threatening or affecting normal activities of the animal because many of these drugs are themselves pro-arrhythmic i.e. they may exacerbate the disturbance or cause some further rhythm complications. Antiarrhythmic therapy is rarely considered essential for occasional premature beats.

Classification

Antiarrhythmic drugs are frequently grouped into 4 main classes according to the Singh Vaughan Williams classification introduced in 1970. In 1979, Harrison proposed a modified sub grouping of class I agents on the basis of their electrophysiological actions. The Singh-VW classification system is arbitrary because many drugs exert multiple effects. Further this system is mainly applicable for drugs used against tachyarrhythmias, and anti-bradyarrhythmics do not fit into this classification. However, the classification system based on 4 major groups is still widely accepted to classify drugs used in arrhythmias.

I. Class I antiarrhythmic drugs: Sodium channel blockers
   1. Class I_A drugs
      e.g. quinidine, procainamide and disopyramide.
   2. Class I_B drugs
      e.g. lidocaine, mexiletine, tocainide, phenytoin and aprindine.
   3. Class I_C drugs
      e.g. flecainide, propafenone, moricizine, encainide and indecainide.

II. Class II antiarrhythmic drugs: Beta-adrenoceptor antagonists
   e.g. propranolol, atenolol, esmolol, sotalol, timolol, carazol and bisoprolol

III. Class III antiarrhythmic drugs: Potassium channel blockers
   e.g. amiodarone, dronedarone, bretylium, bunaftine, ibutilide and nifekalant.

IV. Class IV antiarrhythmic drugs: Calcium channel blockers
   1. Diphenylalkylamine derivatives
      e.g. verapamil and gallopamil.
   2. Benzothiazepine derivatives
      e.g. diltiazem and clentiazem.
   3. Dihydropyridine derivatives
      e.g. nifedipine and amlodipine.

In addition to above mentioned antiarrhythmic drugs, some other drugs which are not basically antiarrhythmics may also be used also in the therapy of arrhythmias. These include cardiac glycosides, adrenoceptor agonists, adenosine and anticholinergics.

I. CLASS I ANTIARRHYTHMIC DRUGS: SODIUM CHANNEL BLOCKERS

Class I antiarrhythmic drugs act by blocking voltage-sensitive sodium channels by the same mechanism as local anaesthetics. Therefore, they possess a membrane stabilising effect resulting in reduced rate of depolarisation (reduced rate of rise of phase 0 of action potential). This also produces a decrease in excitability and conduction velocity. All class I antiarrhythmic drugs show use dependent or state-dependent sodium channel blockade with or without effect on repolarisation.

Mechanism of Action

Class I antiarrhythmics, often called membrane stabilizing anti-arrhythmic drugs, produce their pharmacological effects primarily by blocking myocardial Na^+ channels. They bind to a specific receptor-like site on the sodium ion channel protein and block its function (decrease current). The extent of Na^+ channel blockade is dependent on the heart rate and membrane potential. In general, the class I antiarrhythmic drugs bind more strongly to open or refractory (inactivated) sodium channels and have little affinity for channels in the resting state (after full repolarisation). Further with each action potential, they bind to and block the Na^+ channels, and with each diastolic interval they dissociate and unblock the channels. Therefore, during tachycardia they bind more frequently to the Na^+ channels because increased heart rate increases the frequency of depolarisation and hence frequency of channel opening. Simultaneously, the time required for their unblocking of Na^+ channels during tachycardia decreases (when heart rate increases, the time available for unblocking decreases). These use-dependent actions of class I anti-arrhythmics significantly increase the steady state Na^+ block. The class I antiarrhythmic drugs, therefore, show greater degree of blockade in tissue which are depolarising frequently or which are discharging at abnormally high frequency beating of heart.

Sub-Classification

Class I antiarrhythmic drugs are divided into three sub-groups viz. I_A, I_B, and I_C based upon their varied effects on the action potential (mainly duration of effective refractory period and rate of slowing of phase 0 depolarisation) (Table 33.1). This sub-classification, however, is of little clinical significance. Drugs belonging to subclass I_A and I_B are commonly used in veterinary medicine.

1. Class I_A Antiarrhythmic Drugs

Class I_A antiarrhythmic drugs are sodium channel blockers which moderately slow the rate of rise of action potential (dv/dt) (moderately slow conductivity) and prolong the duration action potential and effective refractory period. These drugs inhibit pacemaker depolarisation and suppress AV conduction. These drugs are mainly useful in ventricular tachyarrhythmias and in prevention of paroxysmal recurrent atrial fibrillation (triggered by vagal over activity).

Quinidine

Quinidine is the oldest antiarrhythmic drug in clinical use and it serves as the prototype Class I_A antiarrhythmic agent. It is an alkaloid obtained from *Cinchona* bark or related plants and is the dextro-isomer of antimalarial drug quinine. It is available commonly in there salt forms: gluconate, polygalacturonate and sulphate.

Mechanism of action

Quinidine blocks myocardial Na^+ channels in a frequency or use dependent manner i.e. at higher heart rates the block increases, while at lower heart rates the block decreases. It has an intermediate speed of association with open or inactivated sodium channels and also an intermediate rate of dissociation from resting channels. This moderately slows the rate of rise of action potential (Phase 0 depolarisation), thus moderately slowing conductivity. Quinidine also prolongs the duration of action potential due to K^+ channel block and lengthens effective refractory period caused by its moderate effect on recovering Na^+ and K^+ channels (Figure 33.7). Due to decreased sodium influx, there is also decreased efflux of potassium that helps in the prolongation of duration of action potential. At high concentrations, quinidine also inhibits L type Ca^{++} channels.

Quinidine decreases automaticity due to decrease of slope of Phase 4 spontaneous depolarisation occurring probably as a result of reduction of diastolic sodium influx. Its Na^+ channel blocking properties may also increase threshold for excitation, which depresses myocardial excitability and also automaticity. Quinidine possesses prominent anticholinergic action (vagolytic action) that antagonises the cardiac action of acetylcholine. It also produces some α-adrenoceptor and Na^+-K^+-ATPase blockade actions.

Pharmacological effects

Heart: Quinidine depresses myocardial conduction velocity, automaticity and excitability, and prolongs duration of action potential and effective refractory period.

Conduction velocity: Quinidine reduces conduction velocity by reducing the rate of Phase 0 depolarisation. This effect is more marked in case of premature action potential; therefore, a premature impulse may fail to propagate. Quinidine also prevents re-entrant arrhythmias by converting a unidirectional block to a bi-directional block through decreasing membrane responsiveness and prolonging the effective refractory period. The anticholinergic activity of quinidine may decrease vagal tone and enhance AV conduction.

Automaticity: Quinidine decreases automaticity in Purkinje fibres and other ectopic foci by decreasing the slope of Phase 4 spontaneous depolarisation and by raising the threshold to electrical stimuli. This serves to extinguish extrasystole and is helpful in treating arrhythmias caused by impulses originating at a rapid rate at ectopic foci. The SA node automaticity is little affected at therapeutic concentration but a 'sick sinus' may be markedly depressed. Quinidine is useful in the prophylaxis of re-entrant arrhythmias but it has little effect on after-depolarisations.

Excitability: Quinidine suppresses excitability by increasing threshold for excitation. It shifts the threshold potential towards zero (less negative); therefore, a greater stimulus is required to initiate an action potential. This action may help to terminate rapid atrial and ventricular rhythms, and cause a conversion to regular sinus rhythm.

Refractory period: Quinidine prolongs the effective refractory period (ERP) and action potential duration (APD). The ERP is prolonged more than APD so that ERP/APD is increased. Thus, tissue remains refractory even after full repolarisation (a class III property). On the AV node, quinidine increases the refractory period by its direct action, but its anticholinergic effect (indirect action) tends to decrease the refractoriness. This antagonistic

action on AV refractoriness may produce either an inconsistent effect or a tachycardia (when indirect vagolytic action predominates over the direct depressant action at the AV junction). Such an adverse effect can be avoided by prior digitalisation of the patient so as to induce a partial but effective AV blockade. Clinically useful doses of quinidine prolongs the effective refractory period in atrial and ventricular muscle with relatively less effect on the refractory period of normal pacemaker cells. Antivagal action adds to this direct action in atrium that serves to terminate atrial fibrillation, atrial flutter and paroxysmal supraventricular tachycardia (normally vagal action shortens the atrial refractory period but prolongs AV refractoriness; vagolytic effect produces opposite effect).

Contractility: Quinidine depresses myocardial contractility, but it is not significant in normal heart. It may precipitate failure in damaged heart.

Electrocardiogram: Quinidine results in prolongation of the QRS complex of ECG due to slowing of conduction velocity in the ventricular muscle fibres. It increases P-R and Q-T intervals. Changes in the shape of T wave may be seen reflecting effect on repolarisation.

Haemodynamics: Haemodynamics are relatively unaffected by low oral doses of quinidine, although negative inotropic effect as a result of interference of transmembrane calcium flux and direct peripheral vasodilatation may cause fall in blood pressure following large oral or intravenous doses. Peripheral vasodilatation occurs mainly due to local anaesthetic and α-adrenoceptor antagonist properties of quinidine.

Other effects: Like quinine, quinidine can decrease contractility of skeletal muscles by direct action. Neurological manifestations may be seen at relatively higher doses. Quinidine also has some antipyretic, antimicrobial and oxytocic actions, although of no clinical significance. Quinidine is bitter and irritant, therefore, it can induce vomiting and diarrhoea.

Pharmacokinetics

Quinidine is nearly completely absorbed from the GI tract after oral administration. The sulphate salt of quinidine is absorbed more rapidly than the gluconate salt of the drug. After absorption, quinidine undergoes hepatic first-pass effect that may decrease amount of drug reaching systemic circulation. After IV administration, quinidine rapidly passes from the blood and distributes into tissues, except the brain. It is highly protein bound (~90%). Serum concentrations are increased and effects enhanced during hypoproteinaemia, metabolic acidosis, hyperkalaemia, etc. Quinidine is metabolised in the liver, mainly by hydroxylation and approximately 20% of a dose may be excreted unchanged within 24 hours after dosing. The serum half-life varies among species and is about 6 hours in dogs and swine, 2 hours in cats, 2.5 hours in cattle, 8 hours in horses and 1 hour in goats. Acidic urine (pH <6) can increase renal excretion of quinidine and decreases its serum half-life. Dissimilar rates of biotransformation probably account for species differences with this drug.

Side effects/Adverse effects

The most common side effects associated with quinidine administration in dogs and cats are anorexia, nausea, vomiting and diarrhoea. In horses, swelling of nasal mucosa, urticarial wheals and laminitis are other potential side effects. More subtle but important side effects related to quinidine include sinus tachycardia, increased ventricular rates in patients with atrial fibrillation, hypotension, syncope and pro-arrhythmic effects. Quinidine over dosage may produce depression, hypotension, lethargy, seizures and cardiac signs (depressed automaticity and conduction or tachyarrhythmias). A 25% increase in the duration of QRS complex, atrioventricular block and acceleration of ventricular tachyarrhythmia are signs of quinidine toxicity. Normally the therapeutic range of quinidine is thought to be 2.5-5 µg/ml, while toxic range is >10 µg/ml in dogs. Monitoring of ECG and serum quinidine concentrations can reduce the likelihood of adverse effects.

Treatment: Treatment of quinidine toxicity includes supportive and symptomatic measures. Intravenous administration of sodium lactate (1/6 M) or sodium bicarbonate may reduce cardiotoxic effects of quinidine by increasing quinidine protein binding. Forced diuresis using fluids and diuretics along with reduction of urinary pH may enhance the renal excretion of quinidine.

Contraindications and precautions

Quinidine is generally contraindicated in patients with complete AV block, intraventricular conduction defect, aberrant ectopic impulses, myasthenia gravis, hepatic impairment and drug sensitivity to it. It should be used with caution in hypokalaemia, hypoxia or acid-base imbalance. Increased toxicity is seen in presence of hypoalbuminaemia. Monitoring of cardiac rhythms in horses is advised.

Drug interactions

Drug interactions are an important consideration during quinidine therapy. Quinidine increases digoxin, amiodarone and verapamil serum concentrations, and enhances negative inotropic and hypotensive effects of beta-adrenoceptor antagonists and calcium antagonists. Cimetidine may decrease the metabolism of quinidine by inhibiting microsomal enzymes, while phenobarbital or phenytoin may induce microsomal enzyme reducing quinidine serum half-life by 50%. Concomitant therapy with other antiarrhythmics usually enhances quinidine effects. Drugs those alkalinise the urine (e.g. sodium bicarbonate, thiazide and carbonic anhydrase inhibitor diuretics) may decrease excretion of quinidine, while acidifying drugs (e.g. ammonium chloride) may increase urinary excretion of quinidine.

Clinical uses

In veterinary medicine, quinidine is used mainly in small animals or equine for the treatment of supraventricular arrhythmias. It is useful in supraventricular arrhythmias associated with anomalous conduction in Wolff-Parkison White (WPW) syndrome and acute atrial fibrillation. It is useful for long-term therapy.

Quinidine is most commonly administered orally. Quinidine can be given IM, but is often painful. Intravenous dosing of quinidine is discouraged because of haemodynamic depression, but it can be used if myocardial performance is normal or only minimally impaired. Oral therapy is generally not preferred in cats. Individualised therapy is necessary because of significant pharmacodynamic variations among animals. The GI upsets may be decreased, if quinidine is administered with food. Oral products of quinidine should be administered at evenly spaced intervals throughout the day/night.

Dose

Dogs & cats : 6 – 20 mg/kg, PO, 3 to 4 times daily.
Cats : 4 – 8 mg/kg, IM, 3 times daily.

Horses : 20 mg/kg, PO, every 2 hours (maximal dose 60 g daily) by stomach tube until arrhythmia is abolished or toxic effects are seen.

: 1 mg/kg, IV (of quinidine gluconate), every 5 to 10 minutes.

Procainamide

Procainamide HCl is a class I_A antiarrhythmic drug that is structurally related to local anaesthetic procaine. It differs from procaine by the substitution of an amide group for the ester group, which protects it from rapid enzymatic hydrolysis and marked CNS effect.

Pharmacological effects

Procainamide has similar mechanism of action and electrophysiological profile to those of quinidine. It affects cardiac automaticity, excitability, responsiveness and conduction similar to quinidine. However, its effects on the autonomic nervous system are significantly weaker. Its vagolytic effects are minimal and also it does not cause α-adrenergic blockade or paradoxical acceleration. It causes somewhat less marked depression of myocardial contractility and AV conduction. Procainamide's effect on heart rate is unpredictable, but it usually causes only slight increase or no change in heart rate. Cardiac output, blood pressure and peripheral vascular resistance are minimally affected by oral doses of procainamide, but large IV doses depress arterial pressure due to ganglionic blockade.

Pharmacokinetics

Procainamide is rapidly and almost completely absorbed after oral administration. In dogs, oral bioavailability is approximately 85% and absorption half-life is 0.5 hours. After IM or IV administration, the onset of effect is practically immediate. Therapeutic plasma concentrations range from 4 to 8 µg/ml. Procainamide is extensively metabolised by the liver to metabolites which are generally inactive in dogs. Dogs, unlike humans, do not acetylate procainamide and hence do not produce the active metabolite N-acetyl procainamide. The elimination half-life of procainamide in dogs in variable, but mostly is reported between 2.5 to 4 hours. Procainamide is mainly excreted in urine both as unchanged drug (~50-70%) and metabolites. Some procainamide is also excreted in the milk.

Side effects/Adverse effects

Side/adverse effects of procainamide are similar to quinidine and are seen on GI tract (anorexia, nausea, vomiting and diarrhoea) and heart (inotropism, AV block and multiform ventricular tachycardias). Agranulocytosis and elevated antinuclear antibody titres have been reported following prolonged (2-3 months) therapy in dogs. Procainamide overdosage may produce widening of QRS complex, junctional tachycardia, ventricular fibrillation or intraventricular conduction delays. Hyperkalaemia enhances procainamide's effects.

Contraindications and precautions

Procainamide is contraindicated in patients hypersensitive to it or procaine. It should not be used in patients with 2nd or 3rd degree block heart block or with *torsades de pointes*. Procainamide should be used with caution in patients with significant hepatic or renal disease or with CHF.

Drug interactions

Procainamide should be used cautiously with other antiarrhythmic drugs as their combination may enhance cardiotoxic and other toxic effects. Cimetidine prolongs procainamide's elimination by inhibiting renal clearance.

Clinical uses

Procainamide is indicated mainly for the treatment of ventricular arrhythmias such as frequent ventricular premature depolarisations or ventricular tachycardia. Its actions parallel to those of quinidine and are considered useful in animals which have not responded to quinidine therapy. Although procainamide can be used in high doses in the treatment of supraventricular tachycardias, it is generally more effective in controlling ventricular arrhythmias than atrial arrhythmias. Procainamide is considered as the drug of choice for the maintenance therapy of ventricular arrhythmias in dogs. However, it should not be used to treat ventricular arrhythmias in dogs and cats with AV block. Procainamide is administered intravenously, intramuscularly or orally. When administered intravenously in dogs, a loading dose may first be given, though care should be taken not to cause hypotension.

Dose

Dogs : 8 – 30 mg/kg, PO, 4 times daily.

: 8 – 20 mg/kg, IM, 4 times daily.

: 2 – 8 mg/kg, slow IV over 5 minutes, then 10 – 40 µg/kg/minute IV infusion.

Horses : 25 – 35 mg/kg, PO, 3 times daily.

: 0.5 mg/kg, IV, every 10 minutes until resolution or until a total of 2-4 mg/kg has been given.

Disopyramide

Disopyramide phosphate is structurally dissimilar from other available antiarrhythmic drugs. It is a quinidine like class I_A drug that has prominent cardiac depressant and anticholinergic actions, but no α-adrenoceptor blocking property. Disopyramide usually has no effect on sinus rate because of opposing direct depressant and indirect antivagal actions. Although it is effective for treating supraventricular and ventricular arrhythmias, disopyramide is not routinely used in veterinary medicine because of its relatively rapid elimination and short-half life (<2 hours) in dogs and cats and profound myocardial depressant action. Disopyramide is generally considered to be a 2nd or 3rd line agent for veterinary use.

Dose

Dogs : 6 –15 mg/kg, PO, 3 to 4 times daily.

Other drugs

Other less commonly used class I_A antiarrhythmic drugs are ajmaline, prajmaline, lorajmine and sparteine. These drugs are plant alkaloids derived mainly from natural sources.

2. Class I_B Antiarrhythmic Drugs

Class I_B antiarrhythmic drugs are sodium channel blockers which have little effect on the rate of depolarisation (little decrease in dv/dt of Phase 0) but they decrease the duration of action potential and shorten refractory period. These drugs do not depress AV conduction; therefore they are used in ventricular arrhythmias.

Lidocaine

Lidocaine (Lignocaine) HCl is a local anaesthetic and the prototypic class I_B antiarrhythmic drug. It is used predominantly

for emergency treatment of ventricular arrhythmias when a rapid onset of antiarrhythmic action is required. It is effective only when administered intravenously.

Mechanism of action

Lidocaine directly interacts with the open or inactivated (preferably) sodium channels, and is relatively selective for partially depolarised cells and those with longer action potential duration. Unlike quinidine, lidocaine associates and dissociates rapidly from sodium channels within the time frame of normal beat. Thus it binds rapidly to open channels during Phase 0 of the action potential without having significant effect on the rate of depolarisation, and then unbinds in the time for next action potential, if the cardiac rhythm is normal. Accordingly, a premature beat is aborted because the channels remain blocked during the action potential. Unlike class I_A drugs, lidocaine and other class I_B drugs decrease the duration of action potential and effective refractory period by shortening the repolarisation (Figure 33.7).

Lidocaine is not useful in atrial arrhythmias, probably because atrial action potentials are so short that the Na^+ channels remain in inactivated state only briefly and the diastolic (recovery) times are relatively large. Its preferential block of refractory channels, for example, in ischemia makes it useful in control of ventricular arrhythmias following myocardial infarction.

Pharmacological effects

Lidocaine varies considerably from quinidine and procainamide in its electrophysiological profile. It produces little effect on sinus node, atrial muscle or atrioventricular node, but markedly suppresses automaticity in Purkinje fibres. The rate of Phase 0 depolarisation and conduction velocity in AV bundle or ventricular muscle is not decreased but rather it may improve conduction in depolarised or stretched Purkinje fibres by increasing resting membrane potential to 'near normal values'. As a result of improved conduction, normal transmission is restored in areas of unidirectional block.

Lidocaine markedly decreases the action potential duration (APD) in Purkinje fibres and ventricular muscles, but the effective refractory period (ERP) is reduced to a lesser extent. Thus, ERP/APD ratio is increased. It has practically no effect on APD and ERP of atrial fibres. Atrial re-entry in not affected, whereas ventricular re-entrant tachycardia is suppressed by lidocaine. At therapeutic doses, lidocaine causes either a decrease or no change in membrane responsiveness and excitability. Lidocaine has minimal effect on ECG; QRS broadening does not occur and QT interval may decrease indicting shortening of APD.

Lidocaine has minimal effect on autonomic nervous system. There are no significant changes in cardiac contractility, cardiac output, arterial blood pressure or peripheral vascular resistance during lidocaine therapy. Heart rate occasionally increases following large intravenous boluses or during lidocaine infusion, presumably owing to sympathetic activation caused by peripheral vasodilatation (local anaesthetic effect).

Pharmacokinetics

Lidocaine is well absorbed after oral administration, but efficient first-pass metabolism causes only one-third of the drug to reach systemic circulation. Therefore, lidocaine is almost always given by IV route. Following a therapeutic IV bolus dose, the onset of action is generally seen within 2 minutes that lasts for duration of 10 to 20 minutes. If a constant IV infusion is begun without an initial IV bolus, therapeutic levels (2 to 6 µg/ml) of drug are achieved late (~1 hour). Although absorption of lidocaine after IM administration is also complete, but the monitoring and adjustment of dosages are difficult; therefore IM is reserved for emergency cases where IV administration is not possible. After IV injection, distribution of lidocaine to tissues is rapid. It has a high affinity of fat and adipose tissue and is bound to plasma proteins, primarily alpha$_1$-acid glycoprotein. Plasma protein binding is highly variable and concentration dependent in dogs.

Lidocaine is biotransformed extensively (~90%) by the liver to metabolites, some of which are active. Half-life of lidocaine in dogs is reported to be less than 1 hour. Hepatic disease and reduced hepatic blood prolong lidocaine half-life and its pharmacological effect.

Side effects/Adverse effects

Side/Adverse effects of lidocaine are dose-related and relatively less at therapeutic doses. Adverse effects are usually manifested by central nervous system dysfunction including drowsiness, hypersalivation, ataxia, nervousness, nausea, vomiting and nystagmus. Drowsiness or agitation may progress to muscle twitching, respiratory arrest and convulsions at high plasma concentrations. Hypotension may occur if the IV bolus is given too rapidly. Cats are more susceptible to the CNS and neurotoxic side effects than dogs, requiring reduced IV bolus dosages. Lidocaine may increase ventricular rates and cause reflex sinus tachycardia if used in patients with atrial fibrillation. Lidocaine at very high dosage may induce circulatory collapse with seizures and bradycardia. Large IV bolus may induce sinus arrest, particularly in cats.

Treatment: Lidocaine overdosage should be treated by supportive and symptomatic measures. Mild symptoms may subside by cessation of therapy or reduction of IV infusion rate. Seizures may be treated with diazepam or a short or ultra-short acting barbiturate.

Contraindications and precautions

Lidocaine is contraindicated in patients with known hypersensitivity to the amide-class local anaesthetics. It should not be used in atrial flutter or fibrillation. Lidocaine can aggravate first- or second-degree AV block and is contraindicated during third-degree AV blocking owing to its ability to suppress Purkinje cell automaticity. Lidocaine should be used with caution in patients with liver disease, CHF, shock, hypovolaemia, hypoxia or marked respiratory depression. It should be used carefully in cats, as they are more susceptible to the CNS effects of lidocaine. Its use in patients with Wolff-Parkinson-White syndrome is controversial. Lidocaine is not effective in the presence of hypokalaemia as its electrophysiological effects are dependent on the plasma potassium concentration. Doses should be reduced in CHF or hepatic disease.

Drug interactions

Cimetidine, propranolol and halothane reduce the hepatic clearance of lidocaine and may enhance its levels or effects. Other antiarrhythmic drugs such as procainamide, quinidine, phenytoin and propranolol administered with lidocaine may cause additive or antagonistic cardiac effects. Large doses of lidocaine may enhance suxamethonium-induced apnoea.

Clinical uses

Lidocaine is one of the best antiarrhythmic drugs for most ventricular arrhythmias, principally ventricular tachycardia and

ventricular premature complexes in all species. It is the best first choice antiarrhythmic for life-saving tachyarrhythmias of any cause. Lidocaine is ineffective in atrial arrhythmias. It is also useful in digitalis toxicity because it does not worsen AV block. Besides antiarrhythmic, lidocaine is widely used as a local and topical anaesthetic agent (see Local anaesthetics, Chapter 26). Lidocaine has also been efficient in refractory cases of status epilepticus.

Lidocaine is available only for parenteral administration and is used primarily by IV route. No other drug should be included in the lidocaine IV solution prepared for treatment of cardiac arrhythmias.

Dose

Dogs : 1 – 2 mg/kg, IV bolus, followed by 30 – 50 µg/kg/min, IV infusion.

Cats : 0.25 – 0.75 mg/kg, slow IV, followed by 10 – 40 µg/kg/min IV infusion.

Large animals : 0.25 – 0.5 mg/kg, IV.

Mexiletine

Mexiletine HCl is a structural analogue of lidocaine and possesses similar electrophysiological and haemodynamic profiles. In the Purkinje fibres, it decreases automaticity, shortens action potential, and to a lesser extent decreases effective refractory period. Usually the conduction velocity is unaffected, but may be slowed in patients with pre-existing conduction abnormalities. It has little effect on heart rate, blood pressure and ventricular functions. After oral administration, it undergoes minimal first-pass hepatic metabolism and thus is effective after oral administration. Mexilitine is metabolised in the liver to inactive metabolites.

Mexiletine produces similar and more frequent dose-dependent signs of central nervous system and gastrointestinal toxicity than lidocaine. Nervousness, ataxia, hypersalivation, nausea, vomiting, diarrhoea and seizures can occur following administration of large oral doses of mexiletine. Blood dyscrasias including leucopenia and thrombocytopenia have been documented in dogs. It is contraindicated during bradycardia, AV conduction defects, hypotension, hepatic failure or severe myocardial failure. Drug interactions of mexiletine are similar to those reported for lidocaine. Mexiletine is reported to produce enhanced antiarrhythmic effects when combined with either quinidine or procainamide therapy (significantly better than when used alone). The combined therapy may be more useful in treating refractory ventricular arrhythmias.

Mexiletine HCl is indicated mainly for all types of ventricular tachyarrhythmias, especially frequent ventricular premature beats, ventricular tachycardia, and those induced by digitalis toxicity. Unlike lidocaine, mexiletine is mainly administered by oral route. Administration of mexiletine with meals may alleviate some GI related adverse effects. Mexiletine is not recommended for cats.

Dose

Dogs : 4 – 8 mg/kg, PO, 3 times daily.

: 3 – 5 mg/kg, IV, followed by 5 – 10 µg/kg/min, IV infusion.

Tocainide

Tocainide HCl is a class I_B antiarrhythmic and an amide type local anaesthetic. It is a structural analogue of lidocaine, but instead of a tertiary amine (lidocaine), tocainide is a primary amine that makes it resistant to extensive first-pass metabolism after oral administration. Its mode of action and electrophysiological and haemodynamic profiles are similar to those of lidocaine and mexiletine. Therapeutic concentrations of tocainide in dogs are in the range of 6-10 µg/ml. Like mexiletine, tocainide produces dose-dependent adverse effects including GI and CNS disturbances, hypotension, blood dyscrasias, and also progressive corneal oedema. Pulmonary fibrosis following tocainide therapy has been reported to occur in humans but not in dogs or cats. Drug interaction and contraindications are similar to those reported for mexiletine and lidocaine.

Tocainide has been used in dogs for the long-term control of ventricular arrhythmias which respond to lidocaine. Like mexiletine, tocainide may be combined with other class I antiarrhythmic drugs in treating refractory arrhythmias.

Dose

Dogs : 15 – 20 mg/kg, PO, 3 times daily.

Phenytoin

Phenytoin sodium is an antiepileptic drug with class I_B antiarrhythmic effects. Its cardiac electrophysiological effects are very similar to those of lidocaine. However, unlike lidocaine, phenytoin has limited spectrum of antiarrhythmic activity. Its primary usefulness in veterinary and human medicine is for the management of digitalis-induced arrhythmias because it specifically suppresses digitalis-induced after-depolarisation, and promotes AV conduction when slowed by digitalis. Phenytoin can be administered intravenously or orally. Phenytoin induces liver enzymes and may alter the requirements of other drugs dependent on liver metabolism (see for detail Anticonvulsants, Chapter 22). Toxic manifestations are rare but may include lethargy, ataxia and disorientation. Use of phenytoin in cats is controversial, as cats easily accumulate this drug and develop symptoms of toxicity.

Dose

For ventricular arrhythmias

Dogs : 10 mg/kg, IV, 3 times daily, usually in increments of 2 – 4 mg/kg.

: 30 mg/kg, PO, 3 times daily.

Cats : 2 – 3 mg/kg, PO, once daily.

Aprindine

Aprindine is a class I_B anti-arrhythmic drug with electrophysiological properties similar to those of lidocaine. It appears to have a broader spectrum of anti-arrhythmic activity than lidocaine and is considered effective in controlling pre-mature ventricular beats and ventricular tachycardia. However, it has been found to be less effective against atrial fibrillation, atrial flutter and supraventricular paroxysmal tachycardia. It is less used in human medicine due to potential adverse effects of leucopenia, agranulocytosis and hepatotoxicty, but has been tried in dogs and found useful. It produces dose-related and transient side effects of nausea, vomiting, ataxia, hypotension, seizures and depressed cardiac contractility.

Dose

Dogs : 0.1 mg/kg, IV infusion for 5 min, repeated at 10 min intervals till arrhythmia is controlled. This is followed by 3 mg/kg, PO, 3 times daily.

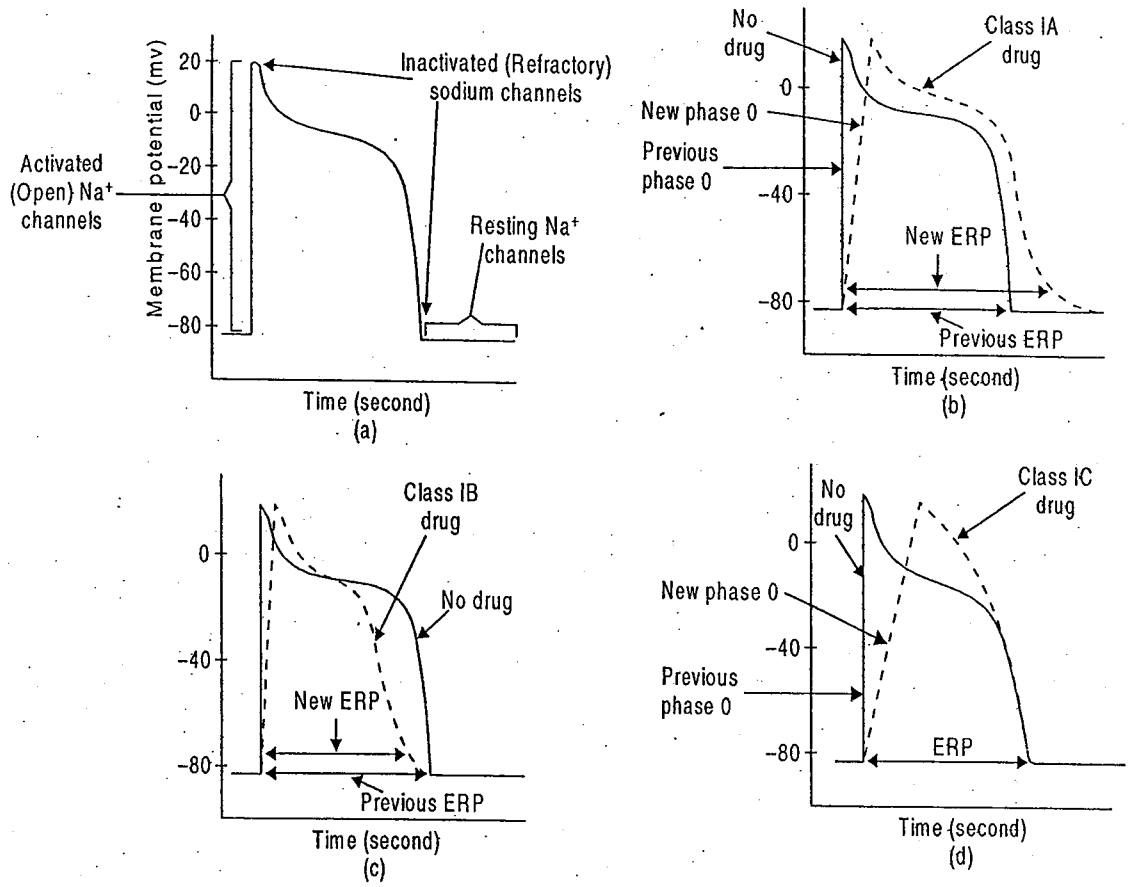


Fig. 33.7. Schematic representation of Class I antiarrhythmic agents.
(a) Normal cardiac action potential showing activated, inactivated and resting sodium channels.
(b) Class IA drugs moderately slow phase 0 depolarisation and prolong ERP and duration of AP.
(c) Class IB drugs marginally slow Phase 0 depolarisation and shorten ERP and duration of AP.
(d) Class IC drugs markedly slow Phase 0 depolarisation, but have marginal effect on ERP and duration of AP.
Abbreviations : ERP = effective refractory period ; AP = action potential.

3. Class I_C Antiarrhythmic Drugs

Class I_C antiarrhythmic drugs are the most potent sodium channel blockers which markedly depress the rate of rise of action potential (marked decrease in dv/dt of Phase 0) and, therefore, cause marked decrease in conduction velocity. However, these drugs have little effect on the duration of action potential or the effective refractory period (Figure 33.7). Class I_C drugs bind slowly to sodium channels to cause their inactivation and hence very long recovery from Na^+ channels. These drugs are less frequently used in veterinary medicine.

Flecainide

Flecainide is a class I_C antiarrhythmic drug occasionally used in human medicine, but has negligible usage in animals. It blocks the sodium channels similar to both class I_A and class I_B antiarrhythmic drugs, but binds slowly and has more prominent action on open state of channels. Flecainide and other class I_C drugs are most noted for their ability to depress the conduction velocity in all parts of heart with greatest effect on the His-Purkinje system and accessory pathways (e.g. Wolff-Parkinson-White syndrome). It has significant haemodynamic effects, which include depression of cardiac contractility, cardiac output and arterial blood pressure.

Important side/adverse of flecainide include depression, nausea, vomiting, hypotension and worsening of CHF. Flecainide and all other class I_C antiarrhythmic drugs possess significant pro-arrhythmic effects and are contraindicated during severe heart failure and pre-existing atrioventricular or bundle branch abnormalities. Flecainide has been suggested as an alternative to quinidine for the conversion of atrial fibrillation in horses, but extensive data on efficacy are lacking. In human medicine, flecainide is used in the treatment of many types of supraventricular tachycardias, including AV nodal re-entrant tachycardia and Wolff-Parkinson-White syndrome in human patients. This is because of the action of flecainide on the His-Purkinje system. Although flecainide is effective in the treatment of both supraventricular and ventricular arrhythmias and arrhythmias caused by accessory pathways (e.g. WPW syndrome), it is not recommended as first choice antiarrhythmic agent.

Dose

Dogs : 5 – 10 mg/kg, PO, 3 times daily.

Other drugs

Propafenone, moricizine, lorcainide, encainide and indecainide are other class I_C antiarrhythmic drugs having properties and adverse effects similar to those of flecainide. Propafenone is

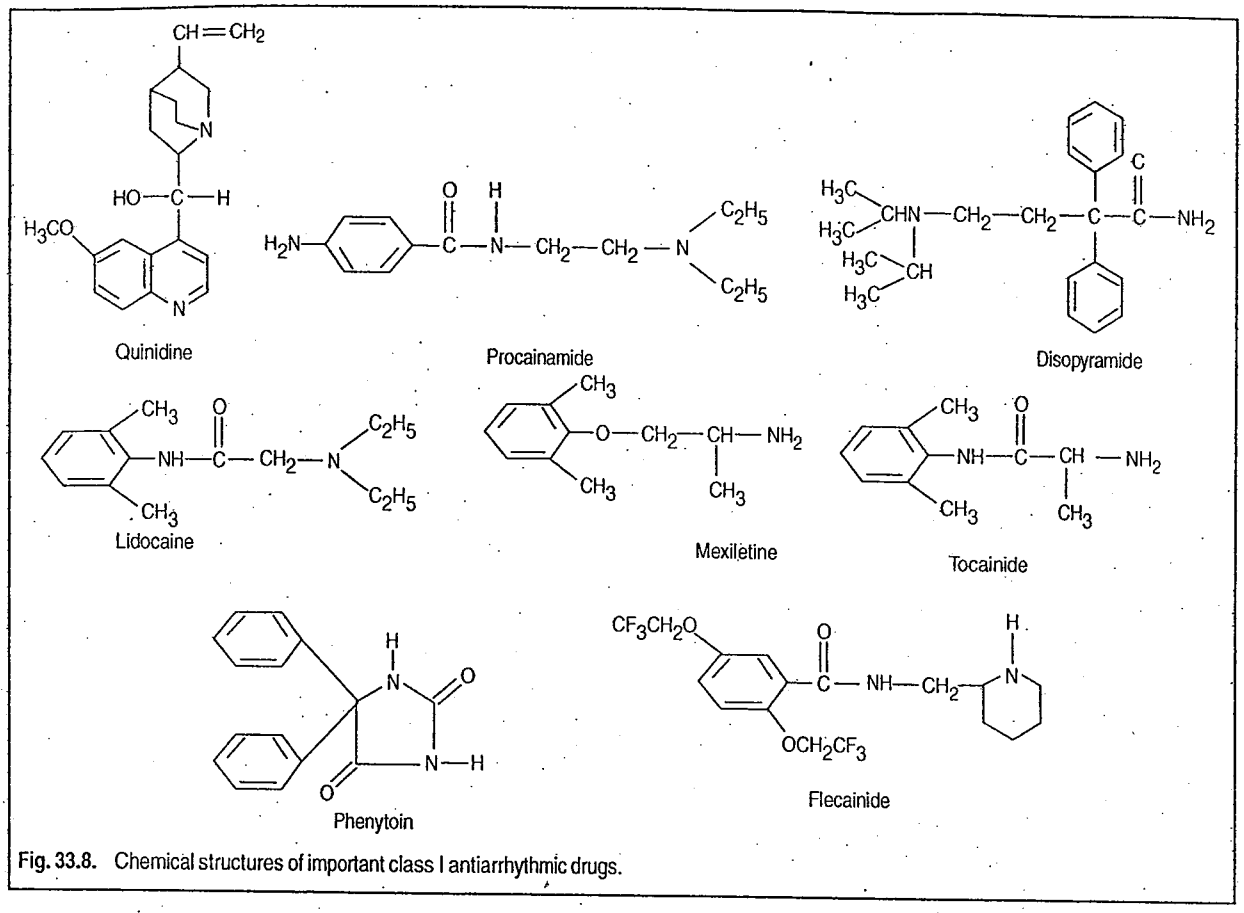


Fig. 33.8. Chemical structures of important class I antiarrhythmic drugs.

clinically used in human medicine for treatment of various types of tachycardia such as atrial and ventricular arrhythmias. Moricizine is slightly less effective than encainide or flecainide in suppressing ventricular premature depolarizations, but it appears to have a low incidence of serious adverse effects compared with other antiarrhythmics. Encainide and indecainide are no longer used because of their frequent proarrhythmic side effects. Like flecainide, these drugs are seldom used in veterinary practice.

II. CLASS II ANTIARRHYTHMIC DRUGS: BETA-ADRENERGIC RECEPTOR ANTAGONISTS

The class II antiarrhythmic drugs are beta-adrenoceptor blocking agents which inhibit sympathetic activity on the heart. They are used mainly to suppress adrenergically mediated arrhythmias.

Mechanism of Action

Beta$_1$-adrenergic receptor stimulation is involved in increasing the magnitude of Ca^{++} current and slowing its inactivation, increasing the magnitude of repolarising K^+ and Ca^{++} currents, increasing the pacemaker current, and under pathophysiological conditions increasing both DAD and EAD-mediated responses. Thus, increased sympathetic nervous system activity not only augments the force of contraction (positive inotropic effect) but also produces unwanted sinus tachycardia, supraventricular and ventricular arrhythmias, and acceleration in ventricular rate during atrial flutter or fibrillation. In heart, beta-adrenoceptor blockers exert antiarrhythmic activity mainly through inhibition of β_1-adrenergic receptors. These drugs inhibit effects of excess sympathetic nervous system by reducing heart rate, decreasing intracellular Ca^{++} overload and inhibiting after-depolarisation mediated automaticity.

Pharmacological Effects

Beta-adrenoceptor blockers decrease the slope of Phase 4 depolarisation and automaticity in SA node, Purkinje fibres and other ectopic foci, particularly when it has been increased under sympathetic influence (little action, otherwise). They also impede AV conduction by prolonging the effective refractory period of AV node. This is useful in terminating re-entrant arrhythmias which involve the AV node and in controlling ventricular responses in atrial fibrillation or flutter. Beta-adrenoceptor antagonists are also considered effective in controlling arrhythmias due to Na^+ channel blockers. Some of the beta-blockers (e.g. propranolol), in addition, have quinidine like direct membrane stabilising action at high doses.

Propranolol

Propranolol is non-selective beta-adrenergic receptors blocking drug and a prototypic Class II antiarrhythmic drug. It is the most common beta-adrenoceptor blocker used in veterinary medicine in conditions of supraventricular tachycardia. It decreases heart rate by a reduction in the sinus node rate and prolongation of AV node conduction. It prevents reflex tachycardia and decreases occurrence of both atrial and ventricular premature depolarisations. Furthermore, it may lower blood pressure in hypertension on prolonged use (see for detail Chapter 12) and reduce deleterious effects of ventricular hypertrophy in conditions like hypertrophic cardiomyopathy, hyperthyroidism and aortic stenosis. Propranolol is a negative inotrope that may be harmful in animals with limited cardiac reserve (e.g. severe CHF), but it is beneficial in cats with hypertrophic cardiomyopathy.

Clinical indications for propranolol as antiarrhythmic drug include reduction of ventricular rate (negative chronotropic effect) in cases of supraventricular tachycardia and atrial fibrillation or flutter in dogs and cats, and as a negative chronotrope and negative inotrope agent in cats with hypertrophic cardiomyopathy. Digitalisation may be necessary before propranolol is used in dogs with CHF. Use of propranolol should be avoided in cats with evidence of respiratory disease (e.g. asthma). Administration of propranolol to animals with little myocardial reserve must be done cautiously. The detailed pharmacology and dosage of propranolol have been given in Chapter 12.

Atenolol

Atenolol is a selective β_1-adrenoceptor blocking drug that may be effective in treating supraventricular tachyarrhythmias, systemic hypertension and hypertrophic cardiomyopathy. It has long duration of action and lacks bronchoconstriction activity (because it lacks β_2-adrenoceptor action). Therefore, it is often useful for long-term therapy in conditions like hypertrophic cardiomyopathy in cats. It requires less frequent dosing than the propranolol (see also Chapter 12).

Esmolol

Esmolol is an ultra-short acting cardio-selective beta$_1$-adrenoceptor blocker. It is also a class II antiarrhythmic with no significant intrinsic sympathomimetic or membrane stabilising activity at therapeutic dosages. It is very useful for the investigation and immediate therapy of tachycardia and is available for intravenous use only (see also Chapter 12).

Sotalol

Sotalol is a non-selective beta-adrenoceptor blocker having a prominent Class III action of prolonging repolarisation by blocking cardiac K^+ channels (therefore, it is sometimes included in class III antiarrhythmic drugs). It does not depress conduction in fast response tissue, but delays AV conduction. It is more effective for many arrhythmias than other beta-blockers, probably because of its additional K^+ channel blocking action. Sotalol is effective in ventricular tachycardia, Wolff-Parkinson-White syndrome, and atrial flutter or fibrillation. Due to prolongation of action potential duration and Q-T interval, the syndrome of *torsade de pointes* is a serious adverse effect.

Other drugs

Some other beta-adrenoceptor blockers commonly used as class II antiarrhythmic drugs include timolol, carazol, acebutolol and bisoprolol. Timolol, nadolol and carazol are non-selective (β_1 and β_2 -receptor), whereas acebutolol and bisoprolol are cardioselective (β_1-receptor) beta-adrenergic receptor blockers.

III. CLASS-III ANTIARRHYTHMIC DRUGS: POTASSIUM CHANNEL BLOCKERS

Class III antiarrhythmic drugs are mostly potassium channel blockers which prolong the process of repolarisation causing widening of action potential duration and effective refractory period without significantly altering Phase 0 of depolarisation or resting membrane potential. These drugs act on all cardiac cells and are used mainly in supraventricular and ventricular tachyarrhythmias. All class III drugs have high potential to induce arrhythmias.

Mechanism of Action

Potassium channels determine pacemaker function, resting membrane potential and action potential duration. The class III antiarrhythmic drugs preferentially block potassium channels and thus diminish outward potassium current during repolarisation of cardiac cells. This increases action potential duration and reduces normal automaticity. The enhanced action potential duration, generally seen as an increase in QT interval, also increases refractoriness (effective refractory period). The tissue remains refractory even after full repolarisation, which helps to terminate re-entrant arrhythmias. Some class III drugs also block inactivated Na^+ channels (e.g. amiodarone) with relatively rapid rate of channel recovery, or myocardial Ca^{++} channels. Few drugs (e.g. sotalol) have important class II beta-adrenoceptor blocking property.

Amiodarone

Amiodarone HCl is an iodine containing highly lipophilic antiarrhythmic drug. Amiodarone is structurally related to thyroid hormone thyroxine. Amiodarone is highly toxic drug, so it should be reserved for life-threatening arrhythmias which are refractory to other drugs.

Mechanism of action

Amiodarone has complex effect showing class I, II, III and IV actions, which have not been fully understood. However, it has been classified as a class III antiarrhythmic drug because of its predominant inhibitory effect on K^+ channels causing prolongation of myocardial cell action potential and effective refractory period. Its other less important effects include inhibition of inactivated Na^+ channels, suppression of myocardial Ca^{++} channels and non-competitive blockade of both alpha- and beta-adrenoceptors. Amiodarone resembles thyroid hormone and its binding to the nuclear thyroid receptor might contribute to some of its pharmacological and toxicological actions

Pharmacological effects

Amiodarone depresses sinus node and auxiliary pacemaker cells automaticity, and in most tissues prolongs the action potential duration and effective refractory period. Amiodarone also appears to reduce non-uniformity of refractoriness among different tissues. Abnormal automaticity (ectopic automaticity) that is dependent on slow channels is significantly inhibited. Amiodarone minimally affects conduction velocity (may be depressed minimally), although it is known to produce mild depression of the fast sodium channels. Amiodarone decreases the force of cardiac contraction, cardiac output and arterial blood pressure following large oral dosage or IV administration. The PR, QRS and QT prolongations and sinus bradycardia (due to decrease in automaticity) are frequent during chronic therapy.

Pharmacokinetics

Amiodarone is administered both orally and parenterally. After absorption, it distributes widely throughout the body and can accumulate in adipose tissue and also heart. It is metabolised mainly in the liver to both active (desmethylamiodarone) and inactive metabolites. It has long half-life of several days in humans. Plasma half-life in dogs after single dosing is about 7.5 hours, which increases to about 3.2 days after repeated dosing. In horses, terminal half-life is 38-84 hours.

Side effects/Adverse effects

Amiodarone produces a wide variety of toxic effects in humans including abnormalities in liver function, sensitivity to sunlight, corneal microdeposits, neurotoxicity and ataxia, thyroid function abnormalities, skin discolouration, pulmonary fibrosis and chronic cough. In dogs, amiodarone mainly produces GI effects (e.g. anorexia and vomiting), but wide spectrum of toxic

effects as seen in humans have not been reported. A high dosage, however, may produce hypotension, bradycardia, cardiogenic shock, AV block and hepatotoxicity. No significant adverse clinical signs are observed in horses after single IV injection. Although amiodarone is less toxic in dogs than in humans, its routine use in small animals is not advised mainly due to its limited experience in animals.

Treatment: Treatment of amiodarone toxicity includes symptomatic and supportive measure like administration of $beta_1$ agonist (e.g. isoprenaline) to counteract bradycardia, and management of hypotension by vasopressor agents or positive inotropic agents.

Contraindications and precautions

Amiodarone is contraindicated in thyrotoxicosis, severe SA node dysfunction with severe sinus bradycardia, 2nd or 3rd degree heart block and hypersensitivity to it. In laboratory animals, amiodarone has shown embryotoxicity at high dosages with congenital thyroid abnormalities in offsprings. As clinical experience in veterinary species is limited, amiodarone should be used with caution in animals.

Drug interactions

Drug interactions are potential problem during amiodarone therapy. The cardiovascular effects of class II and class IV antiarrhythmic drugs are augmented by amiodarone. Concomitant administration of amiodarone with class I_A or class I_B antiarrhythmic drugs may enhance pro-arrhythmic properties of drugs, particularly in presence of hypokalaemia. Quinidine and digoxin blood levels are increased following the initiation of amiodarone therapy. Cimetidine may increase serum levels of amiodarone.

Clinical uses

Amiodarone is used in the treatment of both supraventricular and ventricular arrhythmias in dogs. Because of its toxicity and limited experience, amiodarone should only be considered when recurrent ventricular arrhythmias are not controlled with other therapies. In horses, IV amiodarone has been used to treat atrial fibrillation. Amiodarone is mostly administered in loading doses followed by maintenance doses.

Dose

Dogs	:	10 – 15 mg/kg, PO, 2 times daily for 1 week, then 5 – 7.5 mg/kg, 2 times daily for 2 weeks, followed by maintenance dose of 7.5 mg/kg, once daily.
	:	10 – 20 mg/kg, IV.
Horses	:	5 mg/kg/hr, IV, for 1 hour followed by 0.8 mg/kg/hr for 23 hour.

Dronedarone

Dronedarone is a recently introduced class III antiarrhythmic drug related to amiodarone. However in dronedarone, the iodine moieties are removed to reduce toxic effects on the thyroid and other organs and a methylsulfonamide group is added to decrease lipophilicity and neurotoxic effects. Accordingly, dronedarone possesses less lipophilicity than amiodarone, shows a much smaller volume of distribution and displays an elimination half-life of 24 hours. As a result of these pharmacokinetic characteristics, dronedarone dosing is considered less complicated than amiodarone. Dronedarone is mainly used for the treatment of atrial fibrillation and atrial flutter in human patients whose hearts have either returned to normal rhythm or who undergo drug therapy or electric shock treatment to maintain normal rhythm.

Bretylium

Bretylium tosylate is an adrenergic neuron-blocking drug, now mainly used for its antiarrhythmic effects. Bretylium has complex electrophysiological effects which are partly a result of blockade of norepinephrine release from adrenergic terminals in heart, but major direct action is prolongation of the action potential duration and effective refractory period probably due to K^+ channel blockade. Bretylium has no effect on Na^+ channel, so it does not affect automaticity, conductivity or contractility. Bretylium possesses antifibrillatory effects because it increases the ventricular fibrillation threshold. An initial increase in the release of norepinephrine from adrenergic neuron may result in tachycardia, increased peripheral vascular resistance and possibly a transient worsening of cardiac arrhythmias.

Bretylium is poorly absorbed from the GI tract and therefore, is usually administered parenterally (IV or IM). Important side/adverse effects include hypotension, arrhythmias, ataxia and vomiting. In veterinary and human medicine, bretylium is reserved for life-threatening ventricular arrhythmias, especially those refractory to routine therapy. In pigs, it is used for protection against tachyarrhythmias induced by general anaesthesia. Bretylium has also been discussed in Chapter 12.

Dose

Dogs	:	5 – 20 mg/kg, IV.
Pigs	:	0.02 mg/kg/min, IV infusion.

Sotalol

Sotalol, a class II antiarrhythmic drug, has potent potassium channel blocking effect. The K^+ channel blockade prolongs both repolarisation and duration of action potential, thus lengthening the effective refractory period (see also class II antiarrhythmic drugs).

Other drugs

Other class III anti-arrhythmic drugs occasionally used in human medicine for treatment of various tachyarrhythmias include bunaftine, dofetilide, ibutilide and nifekalant. Some other drugs like tedisamil and vernakalant are currently undergoing clinical trials.

IV. CLASS IV ANTIARRHYTHMIC DRUGS: CALCIUM CHANNEL BLOCKERS

Class IV drugs produce their antiarrhythmic effects by interfering with or blocking the slow voltage sensitive calcium channels. These drugs mainly shorten the plateau phase of action potential and reduce the force of contraction. They have profound effect on atrioventricular nodal conduction and are the drugs of choice for severe acute supraventricular tachycardia. Of the three chemical classes, the diphenylalkylamines (e.g. verapamil) and benzothiazepines (e.g. diltiazem) are mainly used as antiarrhythmic drugs, while the dihydropyridines (e.g. nifedipine and amlodipine) are used clinically as vasodilators (Chapter 34).

Mechanism of Action

Class IV antiarrhythmic drugs primarily inhibit the voltage sensitive calcium channels and decrease influx of Ca^{++} through the cardiac cell membrane into cardiac cell. These drugs bind only to open depolarised channels and prevent repolarisation until the drug dissociates from the channel. They show use-dependent action i.e. they block most efficiently when the heart

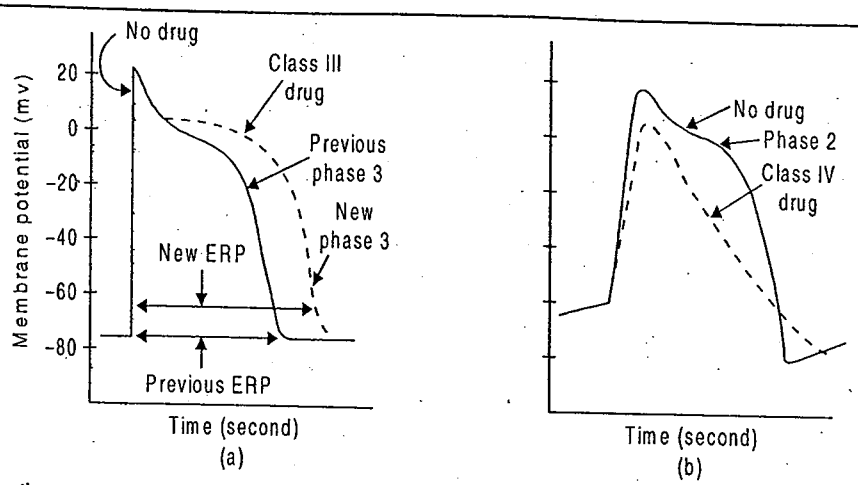


Fig. 33.9. Schematic representation of actions of class III and class IV antiarrhythmic drugs.
(a) Class III drug prolongs phase 3 repolarisation withoutt altering Phase 0 depolarisation. There is marked increase in duration of AP and ERP.
(b) Class IV drug flattens plateau (Phase 2) and slows conduction in tissues dependent on calcium currents such as AV node.
Abbreviations : AP = action potential ; ERP = effective refractory period.

is beating rapidly. They produce less effect in a normally paced heart because the calcium channels have time to repolarise and the bound drug dissociates from the channel before the next action potential. The decrease in the inward current carried by calcium results in decrease in the rate of Phase 4 spontaneous depolarisation and slow conduction in tissues dependent on calcium currents such as SA and AV nodes. These drugs shorten phase 2 (the plateau) of the cardiac action potential (Figure 33.9). Although voltage-sensitive calcium channels occur in many different tissues, the major effect of calcium channel blockers is seen on the L-type Ca^{++} channels located in heart and vascular smooth muscle.

1. Diphenylalkylamine Derivatives

Verapamil

Verapamil HCl is a class IV antiarrhythmic drug that exerts its action by blocking calcium channels in the vascular smooth muscle and myocardial cells. It shows greater action on heart than on vascular smooth muscle cells and is used primarily as an antiarrhythmic drug. It occurs as a bitter tasting nearly white, crystalline powder.

Pharmacological effects

Verapamil depresses calcium-mediated depolarisation and suppresses automaticity in SA node, AV node and Purkinje fibres resulting in suppression of both ectopic and triggered mechanisms. This results in bradycardia and extinction of latent pacemaker. Cardiac arrhythmias caused by oscillatory after-potentials (after-depolarisations) or triggered activities are particularly susceptible to the antiarrhythmic effects on verapamil. The most consistent action of verapamil is prolongation of AV nodal effective refractory period resulting in marked slowing of AV conduction and prolongation of PR interval. This helps in terminating re-entry involving the AV node, and atrial tachyarrhythmias including atrial fibrillation and junction arrhythmia.

Verapamil and other calcium channel blockers decrease intracellular free calcium concentration and reduce the force of cardiac contraction and cause vasodilatation. Reflex sympathetic stimulation due to direct vasodilatory effect partly counteracts the cardiac slowing action of verapamil. Small reduction in cardiac contractile force and vasodilatation may be beneficial in patients with hypertropic cardiomyopathy because of associated decrease in myocardial oxygen consumption and potential decrease in cardiac output due to reduced afterload. However, a negative inotropic effect may exacerbate congestive heart failure.

Pharmacokinetics

Verapamil is rapidly absorbed after oral administration but it undergoes a high first-pass metabolism. It is highly bound to plasma proteins. Verapamil is metabolised in the liver to many metabolites, some of which possess mild antiarrhythmic activity. Verapamil is excreted mainly as metabolites in the urine. Serum half-life of verapamil in dogs following IV and oral administration has been reported to be 0.8 hours and 2.5 hours, respectively. Drugs which reduce liver blood flow or reduce liver enzyme function prolong verapamil elimination.

Side effects/Adverse effects

Important side/adverse effects of verapamil may include sinus bradycardia, atrioventricular conduction disturbances, cardiac depression and hypotension. It may cause anorexia in some patients. Pulmonary oedema, constipation, vomiting and nausea may occur following large oral dosages or long-term oral administration. Very high doses may cause complete AV block and asystole and acute cardiovascular collapse. Intravenous route often produces cardiac depression and hypotension.

Treatment: Treatment of acute verapamil toxicity is generally supportive in nature. Use of fluids and pressor agents (e.g. dopamine and norepinephrine) may be utilised to treat hypotensive symptoms, while IV calcium salts have been suggested to treat cardiac depressant action.

Contraindications and precautions

Verapamil is contraindicated in sick sinus syndrome, cardiogenic shock, severe CHF, AV block, myocardial failure, bradycardia and cardiac glycosides toxicity. It should be used very cautiously in patients with atrial fibrillation, hypertrophic cardiomyopathy and hepatic or renal impairment.

Drug interactions

Verapamil produces additive cardiac depressant action when combined with class I, II or III antiarrhythmic drugs. Intravenous verapamil is contraindicated within a few hours of intravenous beta-adrenoceptor blocking agents as both can depress myocardial

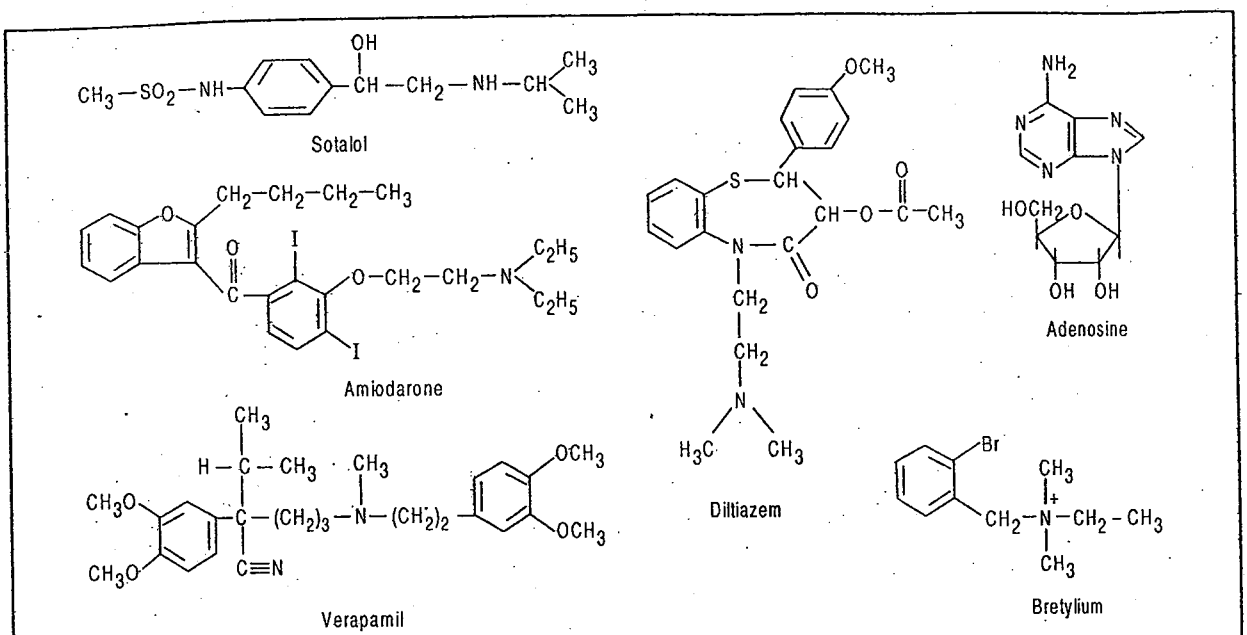


Fig. 33.10. Chemical structures of important antiarrhythmic drugs.

contractility and AV node conduction. It increases cardiac digitalis plasma concentration. Verapamil activity may be adversely affected by vitamin D or calcium salts. Rifampicin may significantly reduce the effects of oral verapamil; these effects may persist for several days after rifampicin is discontinued.

Clinical uses

In veterinary medicine, verapamil is used mainly for supraventricular tachyarrhythmias, sustained and paroxysmal tachycardia, excessive ventricular hypertrophy, and also for the treatment of atrial flutter and fibrillation. Ventricular arrhythmias are generally unresponsive to it. In human medicine it has been used in the treatment of hypertension, angina pectoris, cardiac arrhythmia and cluster headaches. It is also an effective preventive medication for migraine. Overall, the use of verapamil has diminished because of adverse effects.

Verapamil is available for both oral and IV administration. However, it is not recommended routinely by IV route because of the potential for cardiac depression and hypotension.

Dose

Dogs	:	1 - 5 mg/kg, PO, 3 times daily.
	:	0.05 – 0.2 mg/kg, slow IV administered over a period from 2 to 5 minutes followed by IV infusion of 2 – 10 µg/kg/minute.
Cats	:	1 – 3 mg/kg, PO, 3 times daily.

Gallopamil

Gallopamil is an analogue of verapamil. Similar to verapamil it acts on a L-type calcium channel and blocks influx of calcium ions. It is used in the treatment of cardiac arrhythmias.

2. Benzothiazepine Derivatives

Diltiazem

Diltiazem HCl is a benzothiazepine derivative class IV antiarrhythmic drug having Ca^{++} channel blocking activity in both myocardial and smooth muscle cells. Its calcium channel blocking in heart is weaker than that of verapamil but more potent than that of nifedipine. It is used in the treatment of hypertension, angina pectoris and some types of arrhythmia.

Pharmacological effects

Diltiazem prevents the transmembrane influx of extracellular calcium ions in myocardial cells and vascular smooth muscle and produces vasodilatation, negative chronotropic negative inotropic and negative dromotropic effects. Diltiazem causes a modest decrease in heart muscle contractility (negative inotropic) and reduces myocardium oxygen consumption. It causes a modest lowering of heart rate (negative chronotropic) due to slowing of the SA (sinoatrial) node. Similar to verapamil, diltiazem slows conduction (negative dromotropic effect) and prolongs effective refractory period in the AV node. However, it rarely affects SA node conduction, except in patients with sick sinus syndrome where heart rates may be reduced. Diltiazem dilates main systemic and coronary arteries and reduces total peripheral resistance, blood pressure and cardiac after load.

Pharmacokinetics

Similar to verapamil, diltiazem undergoes extensive first-pass hepatic metabolism after oral administration. Some of its metabolites are active and exert Ca^{++} channel blocking action. Half-life of diltiazem in dogs is about 3 hours and in horses is about 1.5 hour.

Side/Adverse effects

Diltiazem is well tolerated by dogs and cats. At normal doses, bradycardia is an important side effect reported in dogs. Hypotension, myocardial depression and AV block are other cardiovascular side effects observed with diltiazem. Non-cardiovascular adverse effects might include GI (constipation and inappetance) or CNS (lethargy) disturbances and increase in liver enzymes.

Contraindications and precautions

Diltiazem is relatively contraindicated in the presence of sick sinus syndrome, atrioventricular node conduction disturbances, bradycardia, impaired left ventricle function, peripheral artery occlusive disease and chronic obstructive pulmonary disease. Diltiazem should not be administered to hypotensive patients. Intravenous administration should always be done slowly. Cardiac rate should be monitored before and during treatment.

Table 33.1: Electrophysiological and pharmacological properties of prototype antiarrhythmic drugs.

Class Conductivity	Drug	Predominant action (Example)	MRD	APD	ERP	Automaticity		AV	Conduction
						SA Node	Ectopic foci		
Class I									
Class-IA	Quinidine	Na^+ Channel block (Moderate association/ dissociation)	⇊	↑	↑	↓/↑	↓	↓	↓
Class-IB	Lidocaine	Na^+ Channel block (Fast association/ dissociation)	↓	↓	↓	0	↓	0, ↑	0
Class-IC	Flecainide	Na^+ Channel block (Slow association/ dissociation)	⇊	0	0			⇊	⇊
Class II	Propranolol	Beta-adrenoceptor antagonism	0	0	0	↓	↓	0, ↓	⇊
Class III	Amiodarone	K^+ Channel block	0	↑↑↑	↑↑↑	↓	↓	↓, 0	0
Class IV	Verapamil	Ca^{++} Channel block	0	↓	0	↓	↓	⇊	⇊

Abbreviations/Symbols: APD = action potential duration; AV = atrio-ventricular; MRD = maximum rate of depolarization (phase 0); ERP = effective refractory period. ↑ = increase; ↓ = decrease: ↓/↑ = variable effect; 0 = insignificant/no effect.

Drug interactions

Intravenous diltiazem should be used with caution with beta-blockers, because while the combination is most therapeutically beneficial, there are rare instances of dysrhythmia and AV node block. Diltiazem and other calcium channel blockers should not be used with quinidine because of reduced clearance of both types of drug and potential pharmacodynamic effects at the SA and AV nodes. Diltiazem IV solution should not be mixed with furosemide. Diltiazem and verapamil inhibit hepatic cytochromes CYP3A4, CYP2C9 and CYP2D6, possibly resulting in drug interactions.

Clinical uses

Diltiazem is indicated for the treatment of atrial fibrillation, supraventricular tachycardias, hypertrophic cardiomyopathy, and systemic hypertension. It is also used to control atrial flutter, AV nodal re-entry arrhythmias, and some other forms of tachycardias. Diltiazem is preferred for long-term therapy due to its less potent negative inotropic effect than verapamil. To slow the ventricular response to supraventricular tachyarrhythmias, generally a lower dosage of diltiazem is initially administered and increased in 2 to 3 days as needed to achieve the desired ventricular rate.

Dose

Dogs : 0.5 – 1.25 mg/kg, PO, 3 to 4 times daily.
: 0.25 mg/kg, IV, over 2 minutes. Dose may be repeated if required.

Cats : 0.5 – 2.5 mg/kg, PO, 2 to 3 times daily.
: 0.15 – 0.25 mg/kg, IV over 2 minutes, and then 1 – 8 µg/kg/min.

Clentiazem

Clentiazem is a chloride derivative of diltiazem. It acts as a calcium channel blocker with activities similar to those of diltiazem.

3. Dihydropyridine Derivatives

Dihydropyridine derivatives consist of many Ca^{++} channel blocking drugs including nifedipine, amlodipine, nicardipine, nitrendipine and felodipine. These drugs have high affinity for vascular calcium channels; therefore, they have a more potent vasodilator effect than the antiarrhythmic action on heart. The pharmacology of these drugs has been discussed in Chapter 34.

MISCELLANEOUS ANTIARRHYTHMIC DRUGS

Since the development of the original Singh Vaughan-Williams classification system, additional anti-arrhythmic agents have been used which do not fit clearly into categories I through IV. Some researchers use the class V anti-arrhythmic drugs for them. However, these drugs with varied mechanisms of action are more frequently taken separately as individual agents.

Cardiac glycosides

The cardiac glycosides have complex pharmacological effect on the heart (Chapter 32). By virtue of prolongation of the effective refractory period of AV node, cardiac glycosides like digoxin control the ventricular response (rate and force) in supraventricular tachycardia, atrial fibrillation and atrial flutter. Digitalis glycosides in high doses are antiarrhythmic and common arrhythmias produced are premature ventricular beats resulting in ventricular tachycardia and fibrillation, AV junctional tachycardia and paroxysmal atrial tachycardia with block. Digitalis glycosides are contraindicated in ventricular arrhythmias. The pharmacology of cardiac glycosides has been described in detail in Chapter 32.

Adenosine

Adenosine (Ado) is a naturally occurring nucleoside, composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety. It modulates many physiological processes through four known adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3), all belonging to the superfamiliy of G-proteins. When administered intravenously, adenosine terminates some types of supraventricular arrhythmias, including any re-entrant arrhythmias that require the AV node for the re-entry (e.g. AV reentrant tachycardia and AV nodal reentrant tachycardia. In addition, atrial tachycardia can sometimes be terminated with

adenosine. At high doses, adenosine decreases conduction velocity, prolongs refractory period and decreases automaticity in the AV node. The activity of adenosine is mediated by the stimulation of specific time-dependent outward potassium current, which appears to be identical to one stimulated by acetylcholine.

Adenosine has an extremely short duration of action (about 15 seconds in man). When administered IV, it is metabolised by adenosine deaminase, which is present in red cells and vessel wall. Adenosine has low toxicity in man, but may cause flushing, chest pain and hypotension. Use of adenosine is contraindicated in 2nd and 3rd degree heart block, sick sinus syndrome, decompensated heart failure, hypotension and asthma. Experience with adenosine in veterinary medicine is limited.

Magnesium

Magnesium sulphate is frequently used in medical and veterinary practices for a variety of conditions. The intravenous administration of magnesium sulphate has been reported to be effective in preventing recurrent episodes of *torsades de pointes*. Intravenous administration of magnesium sulphate is also successful in arrhythmias related to digital intoxication. However, oral Mg^{++} supplements are not useful to produce antiarrhythmic action. The exact mechanism of action of Mg^{++} is not known.

DRUGS FOR BRADYARRHYTHMIAS

Bradyarrhythmia often results from either a failure of impulse initiation or impulse conduction. Failure of impulse initiation may be caused by depressed automaticity resulting from a slowing or failure of phase 4 diastolic depolarization, which may result from disease (e.g. hypoxia and hypothyroidism) or exposure to drugs (e.g. beta adrenoceptor blockers, calcium channel blockers and digitalis). Parasympathetic nervous system (e.g. vagal) over activity also results in bradyarrhythmia. Treatment of bradyarrhythmia is normally aimed at either blocking the overactive vagal effects or improving the slow cardiac activities. For serious and persistent disease, artificial pacemakers may be essential. These should prevent cardiac asystole and therefore prevent sudden death.

Anticholinergic Drugs

Anticholinergic drugs are frequently used to block muscarinic receptors on heart in cases of vagally mediated bradyarrhythmias. These drugs increase heart rate and AV conduction velocity by blocking the parasympathetic neurotransmitter acetylcholine. In veterinary medicine, atropine and glycopyrronium are commonly used for the treatment of bradycardia, incomplete AV block and sino-atrial arrest in dogs and cats. Propantheline bromide can also be effective in bradyarrhythmia and causes fewer side effects than atropine. The pharmacology and other uses of anticholinergic drugs have been discussed in detail in Chapter 14.

Adrenergic Receptor Agonists

Adrenergic receptor agonists like isoprenaline and epinephrine are occasionally used to overcome the partial heart block by facilitating AV conduction and shortening effective refractory period of conducting tissues. These drugs increase heart rate and force of contraction, enhance automaticity and increase conduction velocity by stimulating the cardiac β_1-adrenoceptors. Adrenoceptor agonist drugs are used mainly in emergency treatment of bradyarrhythmias as they precipitate serious tachycardias (see for detail Chapter 11).

CHOICE OF ANTIARRHYTHMIC DRUGS

Choice of antiarrhythmic drugs depends mainly on the electrocardiogram (ECG) diagnosis, possible mechanism of action underlying the arrhythmia, class of the antiarrhythmic drug and homodynamic effects of the drug. Simple arrhythmias in an otherwise normal heart do not require antiarrhythmic therapy, while life threatening arrhythmias require immediate and vigorous therapy. Therefore, an accurate diagnosis of the arrhythmia is essential for selection of an antiarrhythmic drug. Certain guidelines, based on electrophysiological actions of drugs should be followed for selecting an appropriate antiarrhythmic drug.

a. Antiarrhythmic drug should be selected on the basis of type of arrhythmia (Table 33.2).

b. If the initial chosen drug fails, an alternative drug should be used. The alternative drug should have a different electrophysiological action.

c. Drug induced arrhythmias should not be treated with drugs having similar electrophysiological effects.

d. In patients with incomplete AV block, drugs those increase the block must not be used.

Table 33.2: Choice of drugs for cardiac arrhythmias (see also text).

Type of Arrhythmia	Initial Therapy	Alternate Therapy
Tachyarrhythmias		
1. Atrial flutter/fibrillation	Propranolol (β-blockers), diltiazem, verapamil, cardioversion*.	Quinidine, digoxin (digitalis).
2. Supraventricular tachycardia	Propranolol (β-blockers), diltiazem, verapamil.	Digoxin (digitalis), quinidine.
3. Ventricular arrhythmias	Lidocaine, procainamide.	Tocainide, mexiletine, amiodarone, β-blockers.
4. Ventricular fibrillation	Cardioversion*.	Lidocaine, amiodarone, verapamil.
5. Wolff-Parkinson-White syndrome	Cardioversion*, sotalol.	Amiodarone.
Bradyarrhythmias		
1. Bradycardia (Vaso-vagal syndrome)	Atropine.	
2. Partial heart block	Adrenergic agonists.	Atropine.
3. Complete heart block	Artificial pacemaker**	

* Cardioversion: It is an electric shock of a high voltage direct current applied for brief duration through the chest at the level of heart.

** Artificial pacemaker: It is an electrical stimulator that applies electrical shock to the heart and depolarises cardiac muscle to threshold.

e. Drugs having enhanced pro-arrhythmic effects (e.g. class I_C and class III antiarrhythmic drugs) should not be routinely used. If pro-arrhythmic effects are observed or suspected, the selected therapy must be immediately discontinued and alternative therapy initiated.

Combination of Drugs

In the treatment of cardiac arrhythmias, drug combinations should be used only when absolutely necessary. In general, the combination of two drugs with the same type of electrophysiological actions (e.g. quinidine and procainamide) probably has no added advantage. However, certain combinations are rational and useful, if cautiously employed. For example, in supraventricular arrhythmias, digitalis is often employed before quinidine to avoid development of ventricular tachyarrhythmias with quinidine alone. Combination of oral procainamide with intravenous lidocaine is occasionally used, so that when the parenteral drug is stopped, the oral drug continues to provide antiarrhythmic action.

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CHAPTER 34 VASODILATORS AND ANTIHYPERTENSIVE DRUGS

Vasodilators
- Classification
- Arterial vasodilators
  - Direct acting vasodilators
  - Calcium channel blockers
    - Diphenylalkylamines
    - Benzothiazepines
    - Dihydropyridines
  - Potassium channel openers
- Mixed arterial and venous vasodilators
  - Direct acting arterial and venous vasodilators
    - Nitroprusside
    - Organic nitrates
    - Drugs acting on renin-angiotensin system
    - Angiotensin converting enzyme - inhibitors
    - Angiotensin receptor antagonists
    - Drugs acting on adrenergic system
      - Anti-adrenergic drugs
        - Alpha-adrenoceptor antagonists
        - Mixed alpha- and beta- adrenoceptors antagonists
        - Adrenergic neuron blocking drugs
      - Adrenergic agonists
    - Miscellaneous vasodilators
- Inodilators

Antihypertensive drugs
- Hypertension
- Treatment of hypertension
- Vasodilators
- Beta-adrenoceptor blockers
- Diuretics
- Strategies of antihypertensive therapy

VASODILATORS

Vasodilators are drugs which have the ability to cause relaxation of vascular smooth muscle to effect dilatation of blood vessels. These drugs effectively reduce the myocardial workload and may promote cardiac output by increasing the forward stroke volume. Vasodilators are useful in both human and veterinary medicines in the control of hypertension and management of heart failure, where they reduce blood pressure and rate of oedema fluid formation and decrease workload on heart to allow it to work more efficiently with the venous return. In humans, vasodilators are also important for the control of angina pectoris where they improve myocardial perfusion via coronary vessels.

Classification

Vasodilators are arbitrarily divided into three broad categories: arterial vasodilators, mixed arterial and venous vasodilators, and inodilators.

I. Arterial vasodilators
   1. Direct acting arterial vasodilators
      e.g. hydralazine, diazoxide and minoxidil.
   2. Calcium channel blockers
      a. Diphenylalkylamines
         e.g. verapamil.
      b. Benzothiazepines
         e.g. diltiazem.
      c. Dihydropyridines
         e.g. nifedipine, amlodipine, felodipine, isradipine and nicardipine.
   3. Potassium channel openers
      e.g. pinacidil and cromokalim.

II. Mixed arterial and venous vasodilators
   1. Direct arterial and venous vasodilators
      a. Nitroprusside
      b. Organic nitrates
         e.g. glyceryl trinitrate, isoxsuprine and isosorbide.
   2. Drugs acting on renin-angiotensin-aldosterone system.
      a. Angiotensin converting enzyme inhibitors
         e.g. captopril, enalapril, benazepril, fosinopril, lisinopril, perindopril, quinapril, ramipril and trandolapril.
      b. Angiotensin receptor antagonists
         e.g. losartan, candesartan, eprosartan, irbesartan, olmesartan and valsartan.
   3. Drugs acting on adrenergic system
      a. Alpha-adrenoceptor antagonists
         e.g. phenoxybenzamine, phentolamine, prazosin, terazosin and doxazosin.
      b. Mixed alpha- and beta- adrenoceptor antagonists
         e.g. labetalol and carvedilol.
      c. Adrenergic neuron blockin drugs
         e.g. methyldopa, guanethidine and reserpine.
      d. Adrenergic agonists
         e.g. clonidine and guanabenz.
   4. Miscellaneous drugs
      e.g xanthines, papaverine, nicotinic acid, nicergoline, dipyridamole, sildenafil, moxonidine and propentofylline.

III. Inodilators
   e.g. pimobendan and levosimendan

पुस्तक ऋण जारी नहीं

I. ARTERIAL VASODILATORS

Arterial vasodilators (Arteriodilators) act by producing relaxation of arterial (mainly arteriolar) smooth muscle, thereby causing selective arteriodilation. By decreasing the systemic vascular resistance, arterial vasodilators reduce myocardial oxygen consumption, increase cardiac output, and decrease volume of mitral regurgitant flow. Arteriodilators reduce the afterload and have greatest effect in those patients, which have increased systemic vascular resistance as seen in conditions like heart failure, dilated cardiomyopathy and mitral regurgitation.

1. Direct Acting Vasodilators

Hydralazine

Hydralazine (Hydrallazine) HCl is a phthalazine derivative arterial vasodilator that reduces total peripheral resistance with little effect on venous capacitance vessels. It was one of the first active vasodilator/antihypertensive agents marketed.

Mechanism of action

Hydralazine is a direct acting vasodilator. The exact mechanism of vascular smooth muscle relaxant action is not clearly known. It is believed that hydralazine acts by increasing the guanosine monophosphate levels, thereby decreasing the action of the second messenger IP_3 and then limiting the release of calcium from the sarcoplasmic reticulum of smooth muscle. This results in vessel relaxation. The vessel relaxing effect of hydralazine is more significant on arteries and arterioles than on veins. Increase in local prostacyclin concentration, generation of nitric oxide (NO) and direct effects on membrane potential and Ca^{++} fluxes (inhibition) into the cell have also been postulated.

Pharmacological effects

Hydralazine has direct action on vascular smooth muscle with reduced peripheral resistance and blood pressure. It causes greater reduction of diastolic pressure than the systolic blood pressure as it affects mainly the capillary resistance vessels than the post-capillary capacitance vessels. Although hydralazine has no direct action on heart, it reflexly stimulates the heart causing tachycardia and increased cardiac output. The disproportionate cardiac stimulation appears to involve direct augmentation of norepinephrine release and activation of sympathetic nervous system. The renin-angiotensin system can also be stimulated with a resultant increase in sodium and water retention. Reduction in renal blood flow does not occur despite fall in blood pressure. In human patients with acute mitral regurgitation, hydralazine decreases mitral regurgitant flow, increases forward output, reduces left atrial pressure and improves the capacity for exercise. The effects of hydralazine therapy on exercise tolerance and survival in dogs with mitral regurgitation remain unclear.

Pharmacokinetics

Hydralazine is well absorbed after oral administration in dogs, but it undergoes high first-pass effect. The onset of action begins within 1 hour, peaks within 3 hours and remains stable for several hours with a total duration of effect of about 12 hours. It is widely distributed in body tissues and bound to smooth muscle, which results in a biological half-life that is longer than plasma half-life. Hydralazine is metabolized extensively in the liver and approximately 15% is excreted unchanged in the urine.

Side effects/Adverse effects

Important side effects of hydralazine in small animals include hypotension, tachycardia, GI disturbances (vomiting, diarrhoea and anorexia), transient weakness and lethargy and depression. Sodium and water retention may occur if hydralazine is not given concurrently with a diuretic. Reflex tachycardia developing in response to hypotension may be detrimental to the animal with CHF because of increased myocardial oxygen demand. Overdosage of hydralazine may produce toxic effects which are usually extension of its pharmacological effects i.e. severe hypotension, tachycardia and other arrhythmias, and myocardial ischemia.

Treatment: Treatment of hydralazine toxicity involves supportive and symptomatic therapy. Shock may be treated using plasma volume expanders without using pressor agents. If pressor agent is required to maintain blood pressure, the use of a minimally arrhythmogenic agent (e.g. phenylephrine or methoxamine) is recommended.

Contraindications and precautions

Hydralazine is contraindicated in patients with hypovolaemia or pre-existing hypotension, and in those with hypersensitivity to it. It should be used with caution in patients with severe renal and autoimmune disease or in intracerebral bleeding. Hydralazine should be titrated to response of the individual animal.

Drug interactions

Hydralazine should not be used along with sympathomimetics because of additive tachycardia. Concurrent usage of hydralazine with other antihypertensive agents may produce additive hypotension. It may enhance oral absorption of propranolol and other beta-blockers.

Clinical uses

In veterinary medicine, hydralazine is mainly used as an afterload reducer for the adjunctive treatment in congestive heart failure in small animals, particularly if mitral valve insufficiency is the primary cause. It is also used to treat systemic hypertension in small animals. Hydralazine is used both orally (mostly) and parenterally. Because of sodium/water retention and tachycardia associated with this drug, hydralazine is mostly given in combination with a diuretic and beta-adrenoceptor blocker. Many veterinarians recommend using a continuously applied glyceryl trinitrate ointment to reduce the pre-load.

Dose

Dogs	:	0.5 – 2 mg/kg, PO, 2 to 3 times daily. Dose should be gradually increased until a clinical response is elicited or up to a maximum dose of 3 mg/kg, 2 times daily is achieved.
Cats	:	2.5 mg (total), PO, 2 times daily. Dose may be increased to 5 mg (total), 2 times daily, if required.

Diazoxide

Diazoxide is a non-diuretic thiazide that possesses vascular effects similar to those of hydralazine. By acting directly (also through K^+ channel opening) on the smooth muscle of pre-capillary resistance vessels, diazoxide causes vasodilatation and reduces peripheral resistance and blood pressure. The heart rate and cardiac output is increased. Diazoxide also exhibits a potent hyperglycaemic effect, which occurs due to inhibition of pancreatic insulin secretion. On prolonged use, diazoxide leads to diabetes

mellitus hence it is not used as a routine vasodilator. Like hydralazine, diazoxide causes marked Na^+ and water retention when used >1-2 days. In veterinary medicine, diazoxide is used mainly for the treatment of hypoglycaemia secondary to hyperinsulin secretion (e.g. insulinoma), particularly in dogs. As a vasodilator, it is used only to treat malignant hypertension, where it is administered by intravenous route.

Minoxidil

Minoxidil is a powerful arteriodilator that resembles hydralazine and diazoxide in its action. In addition to its direct action, minoxidil enhances K^+ channel opening causing hyperpolarisation of vascular smooth muscle. In human medicine, minoxidil is occasionally used to treat severe hypertension, usually in combination with a diuretic and a beta-adrenoceptor blocker. In humans, it is also often used to treat alopecia areata and androgenic alopecia because of its ability to cause hypertrichosis (growth of hair). The mechanism by which minoxidil promotes hair growth is not fully understood. It is speculated that by widening blood vessels and opening potassium channels, it allows more oxygen blood and nutrients to the follicle causing growth of new hair. Minoxidil has very limited use in veterinary medicine.

Other drugs

Cadralazine, endralazine and dihydralazine are direct acting vasodilators of the hydrazine chemical class. These drugs are used mainly in the treatment of hypertension.

2. Calcium Channel Blockers

Calcium channel blockers are an important class of vasodilator and antihypertensive drugs. These drugs primarily have arterial effects with little to no venodilator effects. As discussed earlier in Chapter 33, three chemical classes of Ca^{++} channel blockers viz. diphenylalkylamine (e.g. verapamil), benzothiazepines (e.g. diltiazem) and dihydropyridines (e.g. amlodipine) are available. The diphenylalkylamines and benzothiazepines affect both cardiac and vascular smooth muscles, but dihydropyridines have a much greater affinity for vascular calcium channels than for calcium channels in the heart, so they are more useful as vasodilators.

a. Diphenylalkylamines

Verapamil

Verapamil is a calcium channel blocker that is valued primarily for its antiarrhythmic effects. Verapamil also relaxes vascular smooth muscles, thereby decreasing peripheral vascular resistance. However, its vasodilatory property is feeble and could be useful only in the control of mild to moderate hypertension. It exerts a significant and detrimental negative inotropic effect in patients with heart failure, which limits its usefulness as a vasodilator/ antihypertensive drug in left ventricular dysfunction. The pharmacology of verapamil has been described in Chapter 33.

b. Benzothiazepines

Diltiazem

Diltiazem is a calcium channel blocker that affects calcium channel in both cardiac muscle and vascular smooth muscle. Like verapamil, diltiazem decreases total peripheral resistance, blood pressure and cardiac afterload due to dilation of pre-capillary resistance vessels. In normal conscious dogs, diltiazem exerts a negligible negative inotropic effect, but manifests chronotropic effect similar to that of verapamil. The use of diltiazem as a vasodilator/antihypertensive drug in small animals has been limited.

c. Dihydropyridines

Dihydropyridine calcium channel blockers include the first generation nifedipine and the newer second generation amlodipine, felodipine, isradipine, lercanidipine, nicardipine, nimodipine, nisoldipine and nitrendipine. All dihydropyridines have much greater affinity for vascular calcium channels than for calcium channels in the heart. They are therefore, more useful as vasodilators than the verapamil and diltiazem. Of various dihydropyridines, amlodipine is commonly recommended for clinical use in veterinary practice.

Nifedipine

Nifedipine is the prototype first generation dihydropyridine calcium channel blocker. Its main uses are as an antianginal and antihypertensive drug in human medicine. It is also used to control premature labour and painful spasms of the oesophagus in cancer and tetanus patients. It has very limited/no usage in veterinary medicine.

Amlodipine

Amlodipine (as besylate, mesylate or maleate) is the prototype second-generation dihydropyridine calcium channel blocker. It has been recommended for use in veterinary medicine for treating systemic hypertension in small animals, especially cats. In human beings it is used mainly an anti-hypertensive and in the treatment of angina. It occurs as a white crystalline powder that is slightly soluble in water and sparingly soluble in alcohol.

Mechanism of action

Amlodipine is a dihydropyridine calcium channel blocker. By binding to the L-type calcium channels in heart and smooth muscles of peripheral vasculature, it inhibits the transmembrane influx of calcium ions into cells. This decreases intracellular calcium concentration that results in relaxation of vascular smooth muscle and myocardium. Amlodipine has a greater effect on calcium channels in vascular smooth muscle (mainly arterioles) than the heart; therefore, it preferentially dilates peripheral blood vessels and reduces after load. The precise mechanisms by which amlodipine relieves angina have not been fully delineated.

Pharmacological effects

Amlodipine lowers blood pressure by relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance. As a consequence of decrease in peripheral vascular resistance, mild to moderate tachycardia resulting from compensatory baroreceptor mediated sympathetic discharge ensues, which acts to attenuate the hypotensive effect of amlodipine. Unlike verapamil and diltiazem, amlodipine does not reduce myocardial contractility (due to feeble effect on heart). Like other Ca^{++} channels blockers, amlodipine also depresses automaticity and conduction velocity in cardiac muscle.

Pharmacokinetics

Pharmacokinetics information on amlodipine in animals is scarce. In humans, amlodipine is slowly but almost completely absorbed after oral administration. It is highly bound (~93%) to plasma proteins; however, drug interactions associated with drug displacement from binding sites have not been elucidated. Amlodipine is slowly but extensively metabolised to inactive compounds in the liver. Amlodipine has long duration of action in humans (half-life = 35 hours), cats and dogs.

Side effects/Adverse effects

Adverse effect profile is not well documented for amlodipine in animals. Limited information suggests minimal adverse effects of amlodipine in cats. Because amlodipine has a slow onset of action, adverse effects such as hypotension and loss of appetite are usually not observed at therapeutic doses. Overdosage of amlodipine may produce profound hypotension and bradycardia. In humans, amlodipine often produces dizziness, palpitations, headache, GI upsets such as dyspepsia and nausea. Blood disorders, gynaecomastia, impotence, depression, insomnia, tachycardia and gingival enlargement are other adverse effects occasionally observed in man.

Treatment: Treatment of amlodipine overdosage is done symptomatically and supportively. Use of beta-adrenoceptor agonists and IV calcium may prove beneficial in amlodipine toxicity.

Contraindication and precautions

Amlodipine should be used cautiously in patients with heart failure, cardiogenic shock, hepatic disease or those at risk of developing hypotension. It is not recommended in dehydrated animals. Amlodipine should be avoided in pregnant animals as it has shown to be foetotoxic in pregnant animals at high dosages.

Drug interactions

Amlodipine shows little interactions with other drugs. Concomitant use of other vasodilator drugs may enhance hypotensive effect of amlodipine. It has no clinically significant drug interactions with other cardiovascular drugs such as cardiac glycosides or warfarin.

Clinical uses

In veterinary medicine, amlodipine is recommended for use in systemic hypertension in cats (mainly) and dogs. In dogs, amlodipine appears to be less effective hence high dosages are required to control hypertension. In man, amlodipine is used clinically in angina pectoris and hypertension.

Dose

Cats : 0.625 – 1.25 mg (total), PO, once daily.

Dogs : 0.05 – 0.25 mg/kg, PO, once daily.

Other dihydropyridine drugs

A large number of dihydropyridine calcium channel blockers with significant vasodilating properties are available for use in human medicine. These agents include aranidipine, azelnidipine, barnidipine, benidipine (benidipinum), clevidipine, efonidipine, felodipine, isradipine, lacidipine, lercanidipine, manidipine, nicardipine, nilvadipine, nimodipine, nisoldipine, nitrendipine and pranidipine. Similar to amlodipine, these agents act by blocking L-type calcium channel in blood vessels and are used as vasodilators to control hypertension. Clinidipine, another dihidropyridine, is the new type of calcium antagonist that act on the N-type calcium-channel that exists in sympathetic nerve end besides acting on L-type calcium-channel similar to most of the dihydropyridine calcium channel blockers.

3. Potassium Channel Openers

Pinacidil

Pinacidil is a cyanoguanidine vasodilator drug. It causes smooth muscle relaxation by selectively increasing the membrane permeability to K^+ thus causing the membrane to hyperpolarize and inhibit action potential. It is believed that pinacidil works by antagonizing the action of intracellular ATP on K^+ channels, thus opening and causing relaxation of these K^+ channels with subsequent vasodilatation. Pinacidil acts mainly at levels of the arteriolar (resistance) vessels causing selective arteriodilation. It is more effective than hydralazine or prazosin.

Pinacidil is used clinically in human medicine and is a promising alternative agent for the treatment of moderate to severe hypertension. Pinacidil also serves a secondary role as an alternative for hair loss treatment. In this capacity, pinacidil, when applied topically, functions in a manner similar to minoxidil and stimulates hair growth. The common side effects of pinacidil are mainly related to vasodilatation, especially oedema, tachycardia, palpitation and flushing.

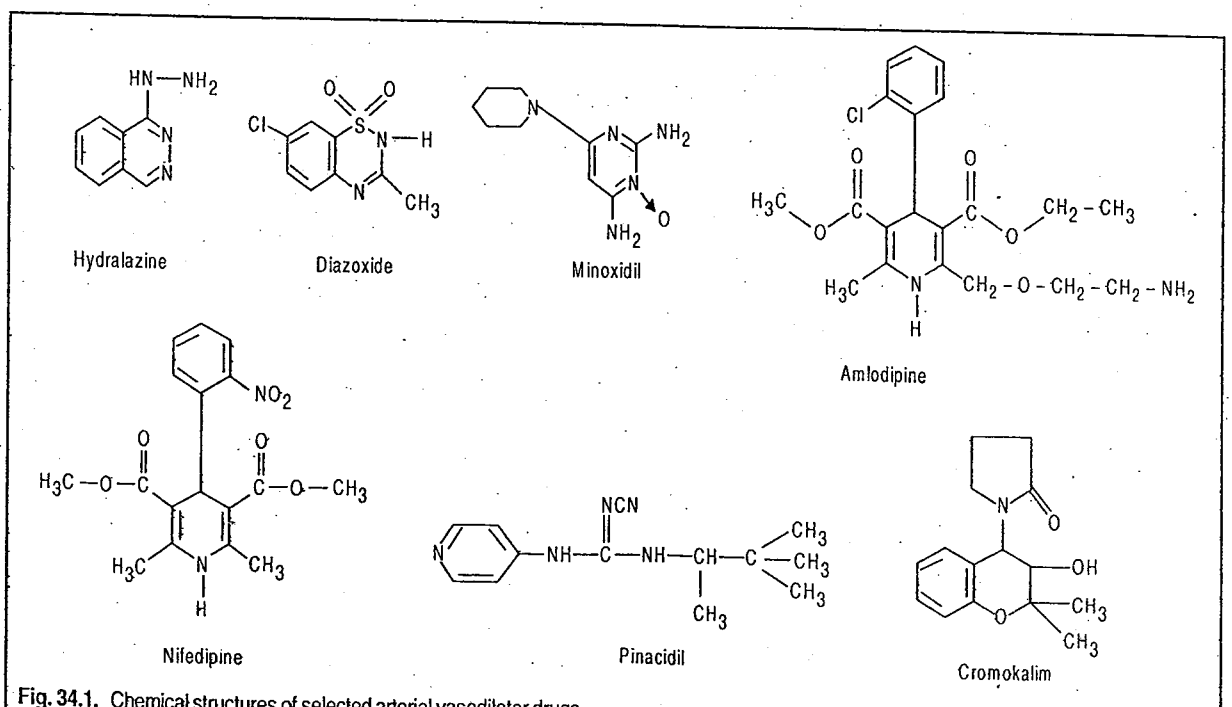


Fig. 34.1. Chemical structures of selected arterial vasodilator drugs.

Cromakalim

Cromakalim is a newly developed K^+ channel activator. It acts on ATP-sensitive potassium channels and so causes membrane hyperpolarization. This leads to decreased activity of vascular smooth muscles causing relaxation. Similar to pinacidil, the vasodilatory action of cromakalim is mainly seen on resistance vessels (e.g. arterioles). Cromakalim is used to treat hypertension and to heart condition. Some tests have shown that cromakalim may be beneficial for hair growth because is works in a fashion similar to minoxidil.

Other potassium channel openers

Levcromakalim is an active enantiomer of the K^+ channel opener cromakalim. It possesses activities similar to those of cromakalim. Diazoxide and minoxidil are believed also to activate K^+ channels in addition to their limited direct action. Clinical use of all these potassium channel openers is limited in veterinary medicine.

II. MIXED ARTERIAL AND VENOUS VASODILATORS

Mixed vasodilator drugs act by relaxing both arterial and venous smooth muscle. They are useful in reducing both pre-load and after-load.

1. Direct Acting Arterial and Venous Vasodilators

A. Nitroprusside

Nitroprusside (Sodium nitroprusside, SNP, nitroferricyanide) is a mixed direct acting vasodilator that acts rapidly and consistently on both arterioles and venules causing prompt vasodilatation. It is one of the most potent vasodilator available that reduces both preload and afterload. It is also capable of reducing blood pressure in all patients and in all conditions, regardless of the cause of hypertension. It is used mainly for the short-term treatment of refractory, life-threatening congestive heart failure in dogs.

Structure and properties

Nitroprusside (Disodium pentacyanonitrosylferrate (2-) dehydrate) is an inorganic compound and a complex anion that features an octahedral ferrous centre surrounded by five tightly bound cyanide ligands and one linear nitric oxide ligand. It occurs as practically odourless reddish brown crystals or powders, freely soluble in water and slightly soluble in alcohol. The reconstituted solutions may have a brownish, straw or light orange colour.

Mechanism of action

Nitroprusside is a nitrate that is composed of a ferrous ion in centre with five cyanide groups and a nitrosyl group. In the circulation, it is metabolised by endothelial cells (and RBCs) to the active metabolite nitric oxide (NO). The nitric oxide activates guanylate cyclase enzyme in vascular smooth muscle and increases intracellular production of cGMP. The cGMP in turn decreases calcium influx and stimulates calcium movement from the cytoplasm to the endoplasmic reticulum and thus reduces calcium available to bind with calmodulin. The increased concentration of cGMP also causes dephosphorylation of myosin light chain kinase (MLCK) through a cGMP dependent kinase to produce relaxation of vascular muscles (see for detail Glyceryl trinitrate). The metabolic activation of nitroprusside is catalysed by a different nitric oxide generating enzyme system than that for glyceryl trinitrate (Figure 34.2). This may be responsible for the different pattern of vasodilatation produced by nitroprusside compared with glyceryl trinitrate.

Pharmacological effects

Intravenous administration of nitroprusside produces rapid relaxation of both resistance and capacitance vessels causing significant reduction in systemic vascular resistance. It produces lowering of blood pressure, venous pooling, reflex tachycardia, and decreased right atrial and pulmonary capillary wedge pressure. In animals with normal left ventricular function, venous pooling affects cardiac output more than reduction of afterload hence cardiac output tends to fall. However in severely impaired left ventricular function and diastolic ventricular distension, the dilation of arterioles and reduction of arterial impudence is the predominant effect leading to improvement of cardiac output. Unlike arteriolar vasodilators, nitroprusside usually causes an overall reduction in myocardial demand for oxygen. Nitroprusside has little action on regional blood flow; renal blood flow and glomerular filtration are maintained and plasma renin activity is increased.

Nitroprusside has little activity outside the vascular system.

Pharmacokinetics

Nitroprusside is administered by constant IV infusion. After starting an IV infusion, reduction in blood pressure begins almost immediately (0.5 minute) and peak hypotensive effects occur within 2 minutes. When IV infusion is stopped, the blood pressure returns to pre-treatment levels within 2 - 10 minutes. Biotransformation of nitroprusside is initiated by smooth muscle by reduction followed by release of 5 cyanide ions and then nitric oxide. Cyanide is further metabolised by liver rhodanese to thiocyanate that is eliminated slowly in urine (mostly), faeces and also in exhaled air. If larger doses are infused for more than 1 to 2 days, excess thiocyanate may accumulate and produce toxicity. The half-life of nitroprusside is 1–2 minutes although thiocyanate has an excretion half life of several days (3 to 7 days) in patients with normal renal function. The elimination half-life may significantly increase in patients with renal insufficiency or with hyponatraemia.

Side effects/Adverse effects

Nitroprusside exerts few adverse effects except for those of hypotension and, with long-term administration, cyanide poisoning. Symptoms such as nausea, retching, palpitation, perspiration, weakness, abdominal pain, disorientation and muscle twitching have been reported in humans and are associated mainly with rapid fall of blood pressure. These adverse effects disappear when the infusion rate is reduced or stopped. The serious consequences of profound hypotension are avoidable when patients are carefully monitored. Toxic accumulation of cyanide radical leading to severe lactic acidosis can also occur if the drug is infused rapidly.

Treatment: Acute overdosage of nitroprusside can be managed by reducing or stopping the infusion and by giving fluids. Hydroxocobalamin (vitamin B_{12a}) may prevent cyanide toxicity. The concomitant administration of sodium thiosulphate (see Chapter 76) can prevent accumulation of cyanide.

Contraindications and precautions

Nitroprusside is contraindicated in patients with hepatic insufficiency, severe renal impairment, hyponatraemia, compensatory hypertension (e.g. AV shunts), shock and inadequate cerebral circulation. To avoid severe hypotension and cyanide toxicity, infusion rate and total dose should be accurately monitored. Solutions should always be prepared fresh and protected from

light during infusion by wrapping the container in aluminium foil or some other light proof material because nitroprusside breaks down to cyanide in light. The reconstituted solution is usually stable for about 24 hours. Mean arterial blood pressure should be critically monitored to avoid excessive hypotension.

Drug interactions

Concomitant administration of other vasodilators, general anaesthetics (e.g. halothane) or ganglionic blocking drugs (e.g trimetaphan) may potentiate the hypotensive effects of nitroprusside.

Clinical uses

Nitroprusside is used clinically in dogs for emergency reduction of blood pressure and for immediate after-load reduction in severe congestive heart failure. It is mostly combined with a positive inotrope such as dobutamine for the management of acute CHF. Dobutamine helps to increase cardiac output and decrease wedge pressure in failing heart. The rate of IV infusion is titrated to effect by monitoring blood pressure and capillary wedge pressure. Despite the toxic potential of cyanide, nitroprusside remains an effective drug in human and veterinary medicines in certain clinical circumstances such as malignant hypertension or for rapid control of blood pressure during vascular surgery and neurosurgery.

Dose

Dogs : 0.5 – 10 µg/kg/min, constant IV infusion. Dose should be gradually increased, if required, maintaining a mean arterial blood pressure above 70 mm Hg. Total dose should not exceed 1.5 mg/kg/2 hour.

B. Organic Nitrates

Organic nitrates are among the oldest and most commonly used drugs for the treatment of cardiovascular diseases. They produce a rapid onset of action causing decrease blood pressure and cardiac filling pressures, and are often used to treat hypertension, pulmonary congestion and episodes of angina.

Glyceryl trinitrate

Glyceryl trinitrate (Nitroglycerine, Nitroglycerol) is an organic nitrate that relaxes vascular smooth muscle. It occurs undiluted as a thick, volatile, white-pale yellow, flammable, explosive liquid with a sweet burning taste. It is an explosive and is used as the major ingredient of dynamite. To produce desired pharmacological effects and to increase its safety, glyceryl trinitrate is diluted with lactose, dextrose, propylene glycol or alcohol. Although it is available in a number of dosage forms, in veterinary medicine glyceryl trinitrate is used mainly topically as an ointment.

Mechanism of action

Like nitroprusside, glyceryl trinitrate (GTN) is a pro-drug that releases nitric oxide (NO) in the vascular smooth muscle. The mechanism by which organic nitrates produce NO is widely disputed and may occur by a variety of mechanisms. It is widely believed that denitrification within the body to produce NO occurs mainly in a reaction with tissue sulphydryl (–SH) groups (Figure 34.2). Some other theories suggest that enzymes such as glutathione S-transferases, cytochrome P450 (CYP) and xanthine oxidoreductase are the primary source of GTN bioactivation. In recent years a great deal of evidence has been produced that support the belief that clinically relevant denitrification of GTN to produce NO is catalysed by mitochondrial aldehyde

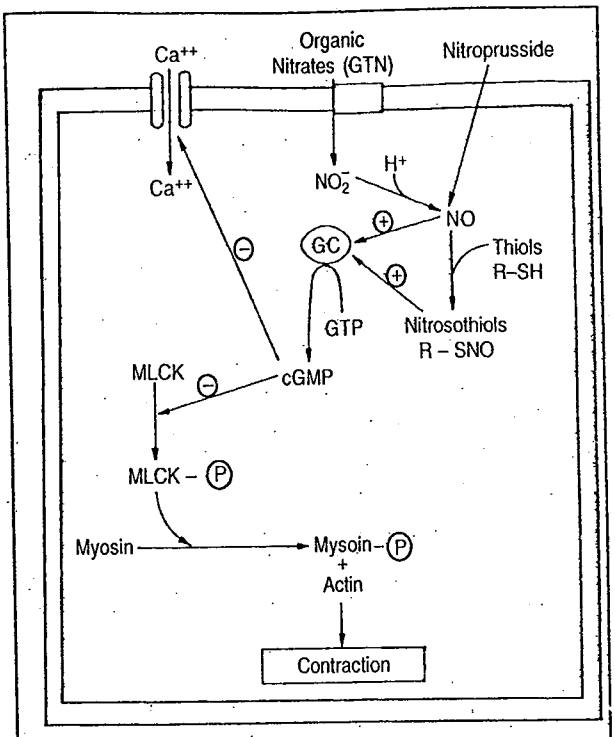


Fig. 34.2. Schematic representation of mechanism of action of nitroprusside and organic nitrates (see text for detail).

Abbreviations : GTN = glyceryl trinitrate ; NO = nitric oxide; MLCK = myosin light chain kinase; GC = guanylate cyclase.

dehydrogenase. Nitric oxide, alone or after formation of a reactive nitrosothiol intermediate, activates a soluble cytosolic form of guanylate cyclase enzyme to produce cGMP. The increased concentration of cGMP causes dephosphorylation of myosin light chain kinase (MLCK) through a cGMP dependent kinase. Reduced availability of phosphorylated MLCK interferes with activation of myosin which prevents the interaction between myosin and actin, thereby resulting in relaxation of vascular smooth muscle. Raised intracellular cGMP may block also the entry of Ca^{++} into the myocyte, which may contribute to vascular relaxation. It appears that sulphydryl (-SH) groups are necessary for production of cGMP in vascular smooth muscle, because depletion of these –SH groups owing to oxidation by nitrates results in tolerance to the effect of these drugs.

Pharmacological effects

Glyceryl trinitrate relaxes vascular smooth muscle primarily on the venous side, but a dose-related effect on pre-capillary vessels may also occur. Preferential venous dilation results in reduction of preload, decreased venous return to heart and peripheral pooling of blood. Myocardial oxygen demand and workload are reduced and coronary circulation may be improved. Preferential mesenteric venous dilatation results in a shift in blood from the pulmonary to systemic vasculature. As glyceryl trinitrate is also an artery vasodilator, afterload may be reduced depending on the dose. Coronary artery vasodilatation might be beneficial to improve perfusion to the hypertrophied myocardium.

Pharmacokinetics

Glyceryl trinitrate is available for IV, sublingual and topical use. Glyceryl trinitrate topical ointment is absorbed through the skin with an onset of action within 1 hour and duration of action of 2 to 12 hours. A transdermal patch in which glyceryl trinitrate

is incorporated into a polymer bonded to adhesive plaster has been developed to provide steady delivery of the drug for 24 hours. Intravenous infusion of glyceryl trinitrate provides rapid, steady and titrable plasma concentration but is used rarely in animals. Glyceryl trinitrate sublingual route (used mainly in human when tablet is crushed under the teeth and spread over buccal mucosa, especially during an attack) provides very rapid onset of action (within 1 to 2 minutes) because the drug is absorbed directly into systemic circulation, bypassing liver where almost 90% of drug is metabolised. Glyceryl trinitrate undergoes extensive but variable fist-pass metabolism in liver. Like nitroprusside, it has very short half-life (1 to 4 minutes); therefore duration of action depends on the period for which the drug remains available for absorption from topical site or buccal mucosa. Glyceryl trinitrate is metabolised mainly in liver to metabolites, some of which have some vasodilatory activity.

Side effects/Adverse effects

Side effects of glyceryl trinitrate are mostly due to vasodilatation and hypotension (mainly orthostatic due to venodilation). Rashes at the site of topical application commonly occur in animals. Severe hypotension may result from excessive absorption of glyceryl trinitrate. After long term use, tolerance to GTN develops that may reduce its effectiveness. The exact mechanism of tolerance development has not been identified, but is believed to be due to impaired biotransformation of GTN to its active metabolite NO (or a NO-related species).

Treatment: If severe hypotension results from topical application of glyceryl trinitrate, the site should be washed thoroughly to prevent any more absorption of drug. Fluids may be administered, if necessary.

Contraindications and precautions

Glyceryl trinitrate and other organic nitrates are contraindicated in liver insufficiency, severe anaemia, cerebral haemorrhage or head trauma, and cardiogenic shock. Glyceryl trinitrate should be used with caution in patients with diuretic induced hypovolaemia or with other hypotensive conditions. Because the drug is absorbed from skin, individuals working with the pets should be alerted to the potential for transcutaneous absorption. Plastic gloves should be worn for application of glyceryl trinitrate.

Drug interactions

Concomitant administration of other vasodilators with glyceryl trinitrate may produce additive hypotensive effects. In human beings, concomitant use of GTN with vasodilators which combat erectile dysfunction, such as sildenafil, has led to severe hypotension, circulatory collapse and death.

Clinical uses

Glyceryl trinitrate is indicated mainly for treatment of acute congestive heart failure particularly that associated with fulminant oedema. It is a useful preload reducer, especially in severe pulmonary oedema. Glyceryl trinitrate is also a coronary artery vasodilator hence it is beneficial to improve perfusion to the hypertrophied myocardium. It is occasionally used in acute laminitis in horses. In humans it is used as antianginal and antihypertensive (acute) agent, and topically in the treatment of Reynaud's disease.

Administration

A 2% ointment of glyceryl trinitrate is most commonly used preparation in veterinary medicine. It is applied to the hairless portion of animal's skin (e.g. abdomen, ear or any other clipped region of body). The inner pinna of ear is used in most cases, unless forward cardiac output is compromised. Topical application of glyceryl trinitrate ointment on the inner pinna of ear reduces chances of exposure to humans and also is less accessible to the patient. Glyceryl trinitrate is typically used for 2-3 days, after which initiation of other myocardial manifestations as well as development of tolerance usually precludes its further use and need.

Dose

Dogs & cats : 0.5 – 4 cm of a 2% topical ointment, 3 times daily.

Isoxsuprine

Isoxsuprine (Isoxuprine) HCl is an organic nitrate that is used as vasodilator in both human and veterinary medicines. It is also a beta-adrenoceptor agonist. Isoxsuprine causes relaxation of vascular and uterine smooth muscle and may have positive inotropic and chronotropic effects on heart. Isoxsuprine induced vascular smooth muscle is most prominent in skeletal muscle (probably due to additional beta$_2$-adrenoceptor agonistic action). Its vasodilating effect is also very significant in certain parts of body such as hooves, feet and brain where it helps increase blood flow and improves circulation.

Tachycardia, hypotension and GI disturbances are common side effects associated with isoxsuprine in horses. It should therefore be used with caution if combined with other drugs which affect blood pressure, such as sedatives and anaesthetic drugs. It should not be administered to animals immediately post-partum, in pregnant mares, or in presence of arterial bleeding. Isoxsuprine is a prohibited class B drug in International Federation for Equestrian Sports- regulated competition, and is often prohibited by other equine associations. Therefore the drug should not be administered to horses before any sporting competition. Sudden withdrawal of drug should be avoided.

Isoxsuprine is primarily used in veterinary medicine mainly to treat hoof-related problems in the horse, most commonly for laminitis and navicular disease. Its effects as a vasodilator are thought to increase circulation within the hoof to help counteract the problems associated with these conditions. In human beings, isoxsuprine is used along with other treatment for certain blood vessel diseases such as arteriosclerosis obliterans, Raynaud's disease (a disorder of the blood vessels, usually of the fingers and toes) and cerebrovascular insufficiency. Isoxsuprine is also a beta-adrenergic agonist that causes direct relaxation of uterine and vascular smooth muscle; therefore, it is used in humans as a tocolytic for treatment of premature labour. Isoxsuprine is given orally and many horses find the pills quite palatable. The drug should be given preferably by addition to a small quantity of feed/suspension on an empty stomach 30 minutes before feeding.

Dose

Horses : 0.6 – 0.9 mg/kg, PO, 2 times daily for 21 days, then once daily for 14 days, then once every other day for 7 days. The dose may be increased to 1.2 mg/kg, 2 times daily for refractory cases. If no improvement is seen after 6-week course, the treatment should be discontinued.

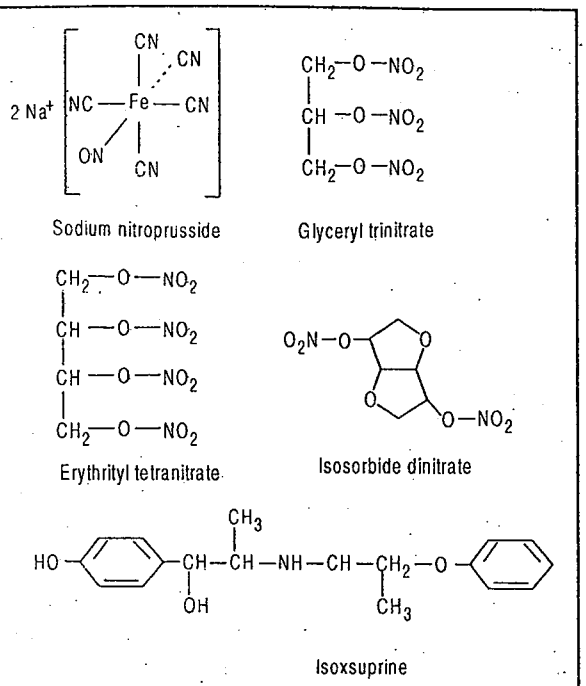


Fig. 34.3. Chemical structures of important mixed arterial and venous vasodilator drugs.

Isosorbide

Isosorbide dinitrate and isosorbide mononitrate, two drugs derived from isosorbide, are used as nitrate vasodilators. Similar to other organic nitrates, they cause vasodilatation primarily by release of nitric oxide in the blood vessels. On oral administration, both drugs are absorbed from GI tract, but the isosorbide dinitrate undergoes hepatic first pass metabolism and it biotransformed to the active form isosorbide mononitrate. Compared to isosorbide dinitrate, the isosorbide mononitrate does not undergo first-pass metabolism and is completely absorbed orally.

Isosorbide dinitrate and isosorbide mononitrate are used mainly in human patients for treatment of angina. In animals, these are occasionally used to reduce pre-load in animals with congestive heart failure. Important side/adverse effects related to their use are primarily related to overdoses which produce vasodilatation and hypotension. Tolerance may develop with repeated use of these drugs.

Dose

Isosorbide dinitrate

Dogs & cats : 2.5 – 5 mg (total), PO, 2 times daily.

Isosorbide mononitrate

Dogs : 5 mg (total), PO, 2 doses daily 7 hours apart.

Other organic nitrate drugs

Several other organic nitrates like erythrityl tetranitrate, pentaerythritol tetranitrate (PETN) and propylene glycol dinitrate (PGDN) are similar in properties to glyceryl trinitrate. These drugs are occasionally used as antianginal drugs in human medicine and have limited use in veterinary medicine.

2. Drugs Acting on Renin-Angiotensin System

a. Angiotensin Converting Enzyme (ACE)-Inhibitors

Angiotensin converting enzyme (ACE) inhibitors are orally active vasodilator/antihypertensive drugs which are used widely in the treatment of chronic congestive heart failure in dogs and cats. These drugs block the angiotensin converting enzyme that cleaves angiotensin I to form the potent vasoconstrictor angiotensin II (see for detail Chapter 28). These agents also diminish the rate of bradykinin inactivation. Peripheral dilatation in both pre-capillary resistance and post-capillary capacitance vessels occurs as a result of the combined effects of lower vasoconstriction caused by diminished levels of angiotensin II and the potent vasodilatory effect caused by increased levels of bradykinin. These agents also diminish the secretion of aldosterone, resulting in decreased sodium and water retention.

The ACE inhibitors lower blood pressure by reducing peripheral vascular resistance without reflexly increasing cardiac output, heart rate or contractility. They reduce both pre-load and afterload in animals with congestive heart. These agents may also help to improve ventricular diastolic function in cardiomyopathies, especially in cats, probably by inhibiting the remodelling effects of angiotensin. Enalapril maleate is the most widely used ACE inhibitors in veterinary medicine, which can delay mortality while improving quality of life in dogs with CHF. Benazepril has been approved recently for treating CHF in dogs. Captopril was the first ACE inhibitor developed for humans and is also used occasionally in animals. The pharmacology of ACE-inhibitors has been described earlier in Chapter 28.

b. Angiotensin Receptor Antagonists

Angiotensin II receptor antagonists are a group of drugs which block AT_1-receptor and modulate the renin-angiotensin-aldosterone system. These drugs include losartan, irbesartan, candesartan, eprosartan, olmesartan, telmisartan valsartan and zolasartan. Unlike ACE-inhibitors, these drugs have no effect on bradykinin metabolism and, therefore, are more selective blocker of angiotensin effects than the ACE inhibitors. Their pharmacological effects are similar to ACE inhibitors in that they produce peripheral vasodilatation and block aldosterone secretion. The angiotensin receptor antagonists have been approved for treatment of hypertension in human medicine (see for detail Chapter 28). There are no reports of their clinical usage in animals.

3. Drugs Acting on Adrenegic System

a. Alpha-Adrenoceptor Antagonists

The conventional non-selective alpha-adrenoceptor blockers like phenoxybenzamine and phentolamine are not routinely used in the treatment of hypertension because fall in total peripheral resistance is compensated by increased heart rate and cardiac output. They are reserved for situation like phaeochromocytoma, where circulating catecholamines are responsible for peripheral vasoconstriction and rise in blood pressure. Use of selective $alpha_1$ adrenoceptor antagonists such as prazosin, terazosin and doxazosin has increased in recent years in therapeutics due to their selective vasodilatation.

Prazosin

Prazosin is a selective $alpha_1$-adrenergic receptor antagonist. It decreases peripheral vascular resistance and lowers blood pressure by causing relaxation of both arterial and venous smooth muscle. It causes minimal changes in cardiac output, renal blood flow and glomerular filtration rate. Therefore, long-term tachycardia and increased renin release do not occur. Prazosin is effective orally, but it undergoes significant first-pass effect and also tolerance to the drug develops rapidly.

Prazosin is occasionally used in veterinary medicine for the adjunctive therapy of CHF, particularly when secondary to aortic or mitral valve insufficiency. It may also be used for the treatment of systemic hypertension or pulmonary hypertension in dogs. The pharmacology of prazosin and other selective alpha$_1$-adrenoceptor blockers is given in Chapter 12.

b. Mixed Alpha- And Beta- Adrenoceptors Antagonists

Labetalol and carvedilol, the mixed alpha- and beta-adrenoceptors antagonists, are occasionally used in hypertensive emergencies. They lower blood pressure primarily by blocking alpha receptors in the peripheral blood vessels, thereby reducing the total peripheral resistance. At the same time, they cause blockade of beta$_1$-adrenoceptor in heart and prevent reflex sympathetic cardiac compensation induced by peripheral vasodilatation. The pharmacology of labetalol and carvedilol has been described in Chapter 12.

c. Adrenergic Neuron Blocking Drugs

Adrenergic neuron blocking drugs such as methyldopa, guanethidine and reserpine are less frequently used in therapeutics to control elevated blood pressure. Methyldopa acts by decreasing the biosynthesis of neurotransmitter norepinephrine (see for detail Chapter 12). Guanethidine and reserpine cause vasodilatation by affecting the release of norepinephrine from adrenergic neurons. Reserpine also blocks the storage of norepinephrine. These drugs are mainly used for the rapid control of blood pressure in a hypertensive emergency. The pharmacology of methyldopa, guanethidine and reserpine has been discussed in Chapter 12.

d. Adrenergic Agonists

The alpha-$_2$ adrenoceptor agonists such as clonidine and guanabenz are occasionally used to treat some disorders of cardiovascular system, especially the hypertension. They control high blood pressure by stimulating α_2 adrenoceptors in the brain, which decrease cardiac output and peripheral vascular resistance leading to lowering of blood pressure. They also have specificity towards the pre-synaptic α_2 receptors in the vasomotor centre in the brainstem and suppress vasomotor tone. The detailed pharmacology of clonidine and guanabenz has been given in Chapter 11.

4. Miscellaneous Vasodilators

Xanthines

The xanthines/methylxanthines, particularly theophylline, dilate systemic blood vessels including coronaries and reduce total peripheral resistance. The effect may be mediated directly or through antagonism of the vasoconstrictor action of endogenous adenosine. Overall the effect on blood pressure is variable and unpredictable because vasomotor centre and direct cardiac stimulation tends to raise blood pressure, whereas vagal stimulation and direct vasodilatation tends to lower blood pressure. Generally a fall in diastolic and rise in systolic blood pressure is observed following methylxanthine administration (see also Chapters 25).

Papaverine

Papaverine is a naturally occurring isoquinoline alkaloid of opium. Pharmacologically, it is quite unlike morphine. Its main action is relaxation of smooth muscle in blood vessels and elsewhere. Its generalised vasodilator effect is not accompanied by tachycardia. The mechanism of action of papaverine is poorly understood, but it seems to involve a combination of phosphodiesterase inhibition and calcium channel blockade. Papaverine is approved to treat spasms of the gastrointestinal tract, bile ducts and ureter and for use as a cerebral and coronary vasodilator. Papaverine may also be used as a smooth muscle relaxant in microsurgery where it is applied directly to blood vessels. Important side effects of papaverine treatment may include ventricular tachycardia, constipation, increased transaminase and alkaline phosphatase levels, and vertigo.

Nicotinic acid

Nicotinic acid (Niacin, vitamin B_3) is a potent vasodilator of superficial blood vessel. It is used occasionally in man to treat problems caused by peripheral vasospasm such as frostbite, chilblain and Raynaud's disease. Nicotinic acid is also a natural medicine widely used for both blood pressure and for lowering LDL cholesterol levels. In humans, high dosage of nicotinic acid can result in a "hot flush", where the skin becomes hot to the point of discomfort.

Nicergoline

Nicergoline is an ergot alkaloid, which acts mainly on the vascular system and cells of the brain. It also blocks alpha$_1$ and alpha$_2$ adrenoceptors, serotonin receptors and dopamine receptors. It decreases vascular resistance and increases arterial blood flow in the brain, improving the utilization of oxygen and glucose by brain cells. It has similar vasoactive properties in other areas of the body, particularly the lungs. It is used mainly for the treatment of age-related problems, particularly behaviour disorders (Chapter 23). In human medicine, it is used to treat senile dementia and other disorders with vascular origins such as cerebral thrombosis and atherosclerosis, arterial blockages in the limbs, Raynaud's disease, vascular migraines and retinopathy.

Dipyridamole

Dipyridamole is a drug that inhibits thrombus formation when given chronically and causes vasodilatation when given at high doses over a short time. It inhibits platelet aggregation by inhibiting thromboxane synthase that causes synthesis of thromboxane A_2 and produces vasodilatation by blocking phosphodiesterase enzyme that normally breaks down cAMP. The vasodilatory effect of dipyridamole is more significantly seen on coronary pre-capillary arterioles which increase total coronary flow. It has no effect on larger conducting coronary vessels. Cardiac output is not affected by dipyridamole because venous return is not reduced. Dipyridamole is considered useful to lower pulmonary hypertension without significant effect on systemic blood pressure.

Sildenafil

Sildenafil and related drugs tadafil and vardenafil are widely used in human medicine to treat erectile dysfunction and pulmonary arterial hypertension (PAH). They act by inhibiting cGMP-specific phosphodiesterase type 5 (PDE-5), an enzyme that regulates metabolism of cGMP in the corpus cavernosum in penis. Increased levels of cGMP leads to smooth muscle relaxation (vasodilatation) and increased inflow of blood into the spongy tissue of the penis, causing an erection. These drugs also decrease pulmonary arterial resistance and pressure and are effective in the pulmonary arterial hypertension (PAH). This, in turn, reduces the workload of the right ventricle of the heart and improves symptoms of right-sided heart failure. Because the PDE-5 is primarily distributed within the arterial wall smooth muscle of the lungs and penis, sildenafil and related drugs act selectively in both these areas without inducing vasodilatation in other areas of the body.

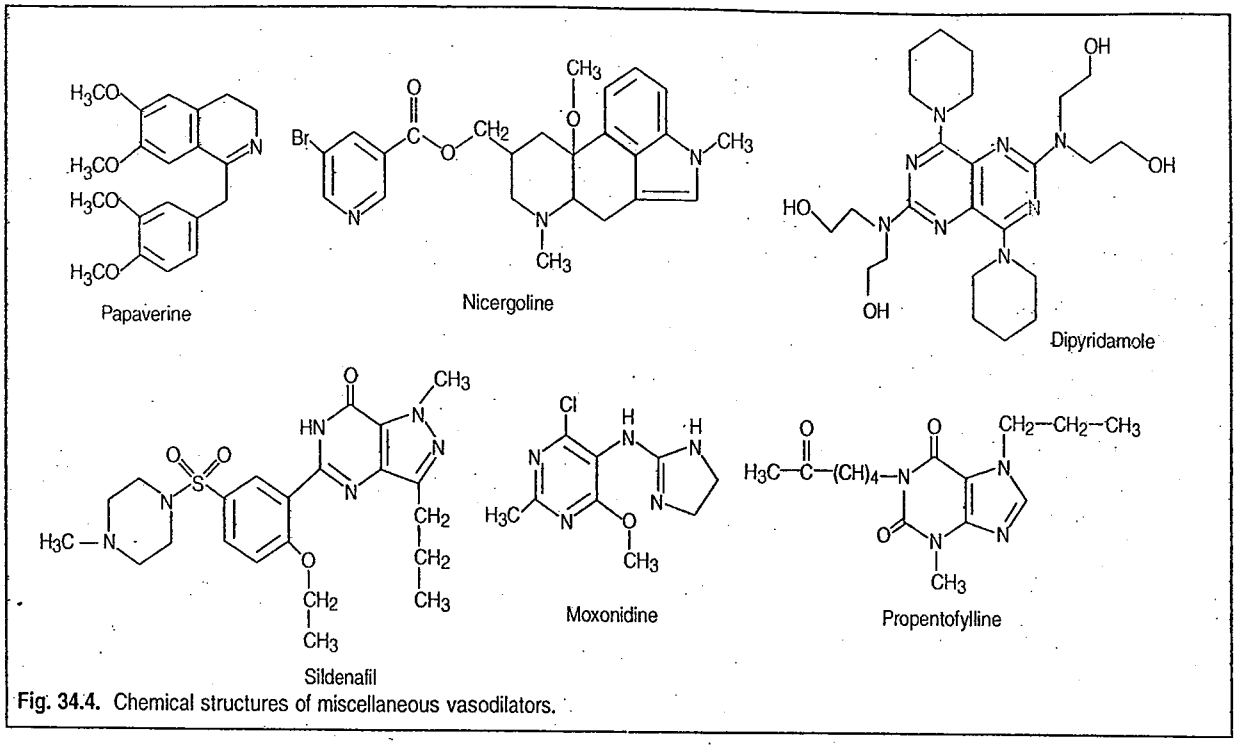


Fig. 34.4. Chemical structures of miscellaneous vasodilators.

The most common adverse effects of sildenafil use in man include headache, flushing, dyspepsia, nasal congestion and impaired vision, including photophobia and blurred vision. Rare but serious adverse effects found through post-marketing surveillance include priapism, severe hypotension, myocardial infarction, ventricular arrhythmias, stroke, increased intraocular pressure and sudden hearing loss. Sildenafil has been tried and found useful in dogs in the end-stage pulmonary artery hypertension. Slow up-gradation in dose in recommended, but may be used in higher dose in acute situations.

Dose

Dogs : 0.25 – 3 mg/kg, PO, 2 times daily.

Moxonidine

Moxonidine is a new generation centrally acting vasodilator drug that acts as a selective agonist at the imidazoline receptor subtype 1. This receptor subtype is found in the medulla oblongata and is involved in the control of central sympathetic tone. Inhibition of imidazoline receptor subtype 1, therefore causes a decrease in sympathetic nervous system activity and hence a decrease in blood pressure. Moxonidine has been approved for the treatment of mild to moderate essential hypertension and is considered useful when thiazides, beta-blockers, ACE inhibitors and calcium channel blockers are not appropriate or have failed to control blood pressure.

Propentofylline

Propentofylline is a xanthine derivative and an inhibitor of phosphodiesterase enzyme. It also acts as an adenosine reuptake inhibitor. Propentofylline is primarily used for neuroprotective effects. It also produces vasodilatation and has been studied as a possible adjunct in the treatment of ischemic stroke. In veterinary medicine, propentofylline is used as a geriatric preparation for old dogs.

III. Inodilators

Inodilation is a relatively new concept in therapeutics where drugs produce enhance myocardial contractility and at the same time produce vasodilatation in peripheral nerves. Two drugs namely pimobendan and levosimendan are available for clinical use. These drugs produce positive inotropic effect mainly by calcium sensitization in the heart, whereas peripheral vasodilatation is produced by inhibition of phosphodiesterase enzyme and thereby increasing cAMP concentration in peripheral vascular cells. The pharmacology of inodilators has been described in Chapter 32.

ANTIHYPERTENSIVE DRUGS

Antihypertensive drugs are a group of pharmaceuticals which are used clinically to lower the elevated blood pressure in systemic hypertension. Systemic hypertension is commonly observed in dogs and cats with chronic renal failure and in a variety of other metabolic and endocrinological abnormalities. Chronic hypertension, either systolic or diastolic, can damage a variety of tissue and can lead to congestive heart failure, renal damage, neurological complications and ocular changes. There is a clear association between ocular injuries (e.g. haemorrhage within the retina, retinal detachment and atrophy, retinal oedema and glaucoma) and marked systemic hypertension in dogs and cats.

Hypertension

Hypertension is a chronic medical condition in which the systemic arterial blood pressure is elevated. It usually refers to a sustained elevated diastolic blood pressure accompanied by an elevated systolic blood pressure.

Types of hypertension: Hypertension is broadly classified as either primary (essential) or secondary. The primary hypertension (also called essential or idiopathic hypertension) is the form of hypertension that has no identifiable cause. It is the most common type of hypertension in humans, affecting 95% of hypertensive patients, but is relatively uncommon in animals. The secondary hypertension results from an identifiable cause such as hormonal imbalance, blood plasma volume alteration, defective cardiac functioning or tumours. Hypertension

can also be classified as either systemic hypertension or pulmonary hypertension on basis of types of blood vessels involved. In the systemic hypertension, there is elevated blood pressure is peripheral and systemic blood vessels. It is more common type of hypertension and the term hypertension generally refers to it. The pulmonary hypertension is related to elevated blood pressure in the lung vasculature (pulmonary artery, pulmonary vein, or pulmonary capillaries). It is less common type but can be a severe disease with a markedly decreased exercise tolerance and heart failure.

Treatment of hypertension

Treatment of hypertension in small animals has been mainly extrapolated from human protocols. As discussed earlier (Chapter 31), the arterial blood pressure is directly proportional to the product of cardiac output and peripheral vascular resistance, which in turn are controlled mainly by two overlapping control mechanisms, the sympathetic nervous system and the renin-angiotensin-aldosterone system. In a hypertensive animal, vascular tone and intravascular pressure may be elevated because of increased sympathetic tone, activation of angiotensin, release of aldosterone, or increased vascular wall resistance caused by salt and water retention. Accordingly, the antihypertensive therapy is generally aimed at reducing cardiac output, total peripheral resistance, extracellular volume, or combination of all.

There are many classes of antihypertensives, which lower essential blood pressure by different mechanisms; among the most important and most widely used are vasodilators, beta-adrenoceptor blockers and diuretics. These agents are used generally in concert with dietary sodium restrictions. A single drug can control mild hypertension, but a more severe hypertension may require combination of drugs, which are often selected to minimise adverse effects of the combined regimen. Secondary hypertension can be managed by treating the underlying cause.

1. Vasodilators

Vasodilators effectively decrease the elevated blood pressure by producing relaxation of vascular smooth muscle and by decreasing total peripheral resistance. In humans with systemic hypertension and renal disease, vasodilator therapy is the preferred initial choice because of the renoprotective effects of certain groups of these agents (e.g. ACE inhibitors and certain Ca^{++} antagonists). Vasodilators include several subclasses of drugs, which have been discussed earlier in this chapter.

2. Beta-Adrenoceptor Blockers

Beta-adrenoceptor blockers exert an antihypertensive effect primarily by reducing the cardiac output. They may also decrease sympathetic outflow from the CNS and inhibit release of renin from the kidneys thus decreasing the formation of angiotensin II and secretion of aldosterone (Figure 34.5). A cardioselective (β_1) antagonist such as atenolol or metoprolol is preferred, while the non-selective prototype beta-blocker like propranolol can also be used. These agents can be combined with vasodilators and/or diuretics. In human medicine, beta-adrenoceptor blockers and/or diuretics are currently recommended as first-line drug therapy for hypertension. The pharmacology of beta-adrenoceptor blockers has been described earlier in Chapter 12.

3. Diuretics

Diuretics reduce blood pressure mainly by lowering extracellular fluid volume and cardiac output (Figure 34.5). Overuse of diuretics, however, can lead to dehydration, volume depletion, and considerable electrolyte imbalance. Hyperkalaemia may occur with loop diuretics as well as thiazides. The addition of spironolactone, a potassium-sparing diuretic may also be helpful in limiting potassium loss. Diuretics such as thiazides may be used in hypertensive dogs and cats. The pharmacology of diuretics has been discussed in Chapter 39.

Strategies of Antihypertensive Therapy

Hypertension is commonly treated with a single or more often a combination of two or more drugs. Following guidelines may help in selection of drugs and in minimizing their adverse effects:

a. Antihypertensive therapy is instituted generally by sequential trials.

b. When using pharmacological agents, a wide range of dosage should be considered with initial dosage at the lower end of the range.

c. Dosage adjustment or changes in drug treatment should not be done frequently (not less than 2 weeks), unless the severe life-threatening hypertension is present.

d. If an agent or combination of agents is not satisfactorily effective, the dosage(s) may be increased or additional agents(s) is added.

e. Periodic dosage adjustment of antihypertensive agent(s) based on blood pressure measurement should be followed for effective therapy.

f. Dietary salt restriction should always be considered as it often enhances efficacy of therapeutic agents.

g. Obesity can elevate systemic blood pressure in humans and dogs, and possible cats. Therefore, weight reduction is desirable in obese, hypertensive animals.

h. Hypertension associated with chronic renal failure often requires life-long antihypertensive treatment with periodic dosage adjustment.

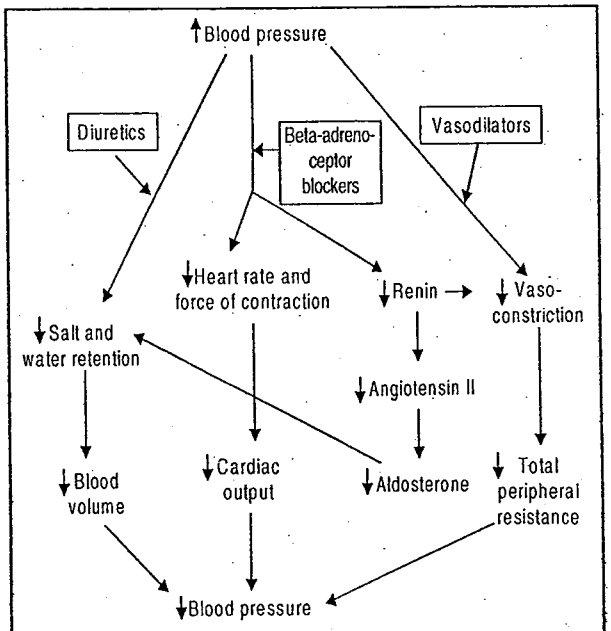


Fig. 34.5. Schematic representation of actions of antihypertensive drugs. Major groups of antihypertensive drugs are shown in boxes.

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CHAPTER 35 DRUGS AFFECTING HAEMOSTASIS AND BLOOD COAGULATION

Haemostasis
Antihaemostasis
  Fibrinolysis
  Inhibitory factors
Haemostatics
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    Coagulants
    Vitamin K and analogues
    Blood and blood components
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Antihaemostatics
  Classification
  Anticoagulants
    In vitro anticoagulants
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      Heparin and related substances
        Heparin and derivatives
        Heparinoids
        Oligosaccharides
      Inhibitors of vitamin K
        Coumarin derivatives
        Indanedione derivatives
      Direct inhibitors of coagulations factors
        Direct inhibitors of factor Xa
          Xabans
        Direct inhibitors of coagulation factor II
          Hirudin and derivatives
          Miscellaneous direct thrombin inhibitors
      Miscellaneous anticoagulants
Thrombolytics/Fibrinolytics
  Plasminogen activators
  Other drugs
Antithrombotics
  Cyclooxygenase inhibitors
  Adenosine diphosphate receptor inhibitors
    Thienopyridines
    Other drugs
  Glycoprotein IIb/IIIa inhibitors
  Thromboxane inhibitors
  Miscellaneous drugs

HAEMOSTASIS

Haemostasis is the prevention or control of blood loss from the damaged blood vessels. It is a complex phenomenon that includes physical and biochemical changes. It starts with the injury to tissue and blood vessels and terminates in the formation of solid blood clot that seals the vascular endothelium. It involves three main processes – vascular contraction, platelet activation and adhesion, and blood coagulation/fibrin formation.

1. Vascular contraction: Physical trauma to the vascular system, such as a puncture or cut, initiates spontaneous localized vasoconstriction, the first step to check the bleeding. Localized vasoconstriction temporarily decreases blood flow and pressure within the ruptured vessel. The contraction of blood vessels results from nervous reflexes, local myogenic spasm and local humoral factors released from traumatised tissues and blood platelets. Pressure of the escaped blood in surrounding tissues and endothelial adhesion also decreases blood escape. This local vascular constriction can last for many minutes or even hours, during which time the ensuing processes of platelet plugging and blood coagulation can take place.

2. Platelet activation and adhesion: Vasoconstriction is rapidly followed by the second step, the mechanical blockage of lumen of the damaged blood vessel by formation of a platelet plug. Normally platelets circulate in blood in an inactive form. When platelets come in contact with a damaged vascular surface, they become activated and change their characteristics drastically. Activated platelets undergo morphological changes to assume irregular forms (pseudopods) with expression of various proteins and receptors. The contractile proteins in the platelet contract forcefully in response to various mediators and cause release of intracellular granules, which contain multiple activating factors including serotonin and adenosine diphosphate (ADP) (Figure 35.1). Stimulation of membrane phospholipases liberates arachidonic acid from membrane phospholipids, which results in synthesis and release of thromboxanes A_2 (TXA_2). The released ADP and TXA_2 in turn act on nearby platelets to activate them as well. The activation of platelets in turn results in the activation of several integrins (receptors which mediate attachment between a cell and the tissues surrounding it) like integrin $\alpha 2\beta 1$ and $\alpha IIb\beta 3$ (commonly called glycoprotein IIb/IIa, gpIIb/IIIa), which then

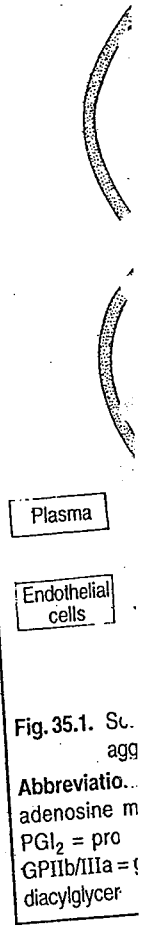


Fig. 35.1. Sc... agg...
Abbreviatio... adenosine m... PGI_2 = pro... GPIIb/IIIa = ... diacylglycer...

mediate the... exposed base...
Aggrega... Willebrand f... bridging co... molecules... molecules a... surface of ... glycoprotein... to a high... signalling... aggregati... at the site... thrombus... cellular ele... arteries ... thrombus... principa... endothelia... platelet ...
3. Pl... initiate th... a series... entire co... solid g...

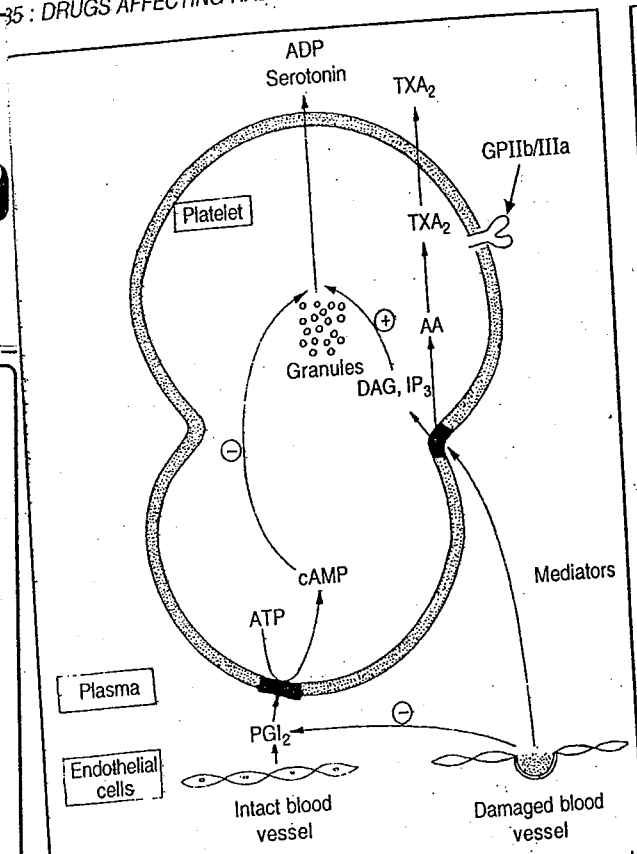


Fig. 35.1. Schematic representation of platelet activation and aggregation (See text for detail).

Abbreviations : ATP = adenosine triphosphate ; cAMP = 3',5'-cyclic adenosine monophosphate ; ADP = adenosine diphosphate ; PGI_2 = prostaglandin I_2/prostacyclin ; AA = arachidonic acid ; GPIIb/IIIa = glycoprotein IIb/IIIa ; TXA_2 = thromboxane A_2 ; DAG = diacylglycerol ; IP_3 = inositol triphosphate ; + = stimulation ; – = inhibition.

mediate the firm adhesion of platelets to each other and with exposed basement membrane, collagen fibres and myofibrils.

Aggregation of platelets is mediated by fibrinogen or Von Willebrand factor (vWf), which connect platelets to each other by bridging complexes of glycoprotein IIb/IIIa (integrin αIIbβ3). molecules on adjacent platelets. The glycoprotein IIb/IIIa molecules are found in large number (50,000 – 80,000) on the surface of platelets. In order to bind fibrinogen and vWf, the glycoprotein IIb/IIIa needs to be converted from low affinity state to a high affinity state by a process described as inside-out signalling that is initiated during platelet activation. The aggregation of platelets forms a loose plug or platelet thrombus at the site of injury. Each thrombus is composed of a white thrombus (platelets) surrounded by a red thrombus (fibrin and cellular elements). Although all thrombi are of mixed type, in arteries platelet mass (white thrombus) is the main constituent of thrombus and in veins red thrombus with a fibrin rail is the principal part mainly due to sluggish blood flow. Damaged endothelial cells also synthesise less of prostacyclin, which favours platelet activation and adhesion.

3. Blood coagulation: The platelet activation and adhesion initiate the third step, the blood coagulation. This occurs through a series of reactions known as the coagulation cascade. The entire coagulation process or cascade converts fluid blood to a solid gel or clot through two interrelated pathways - the extrinsic

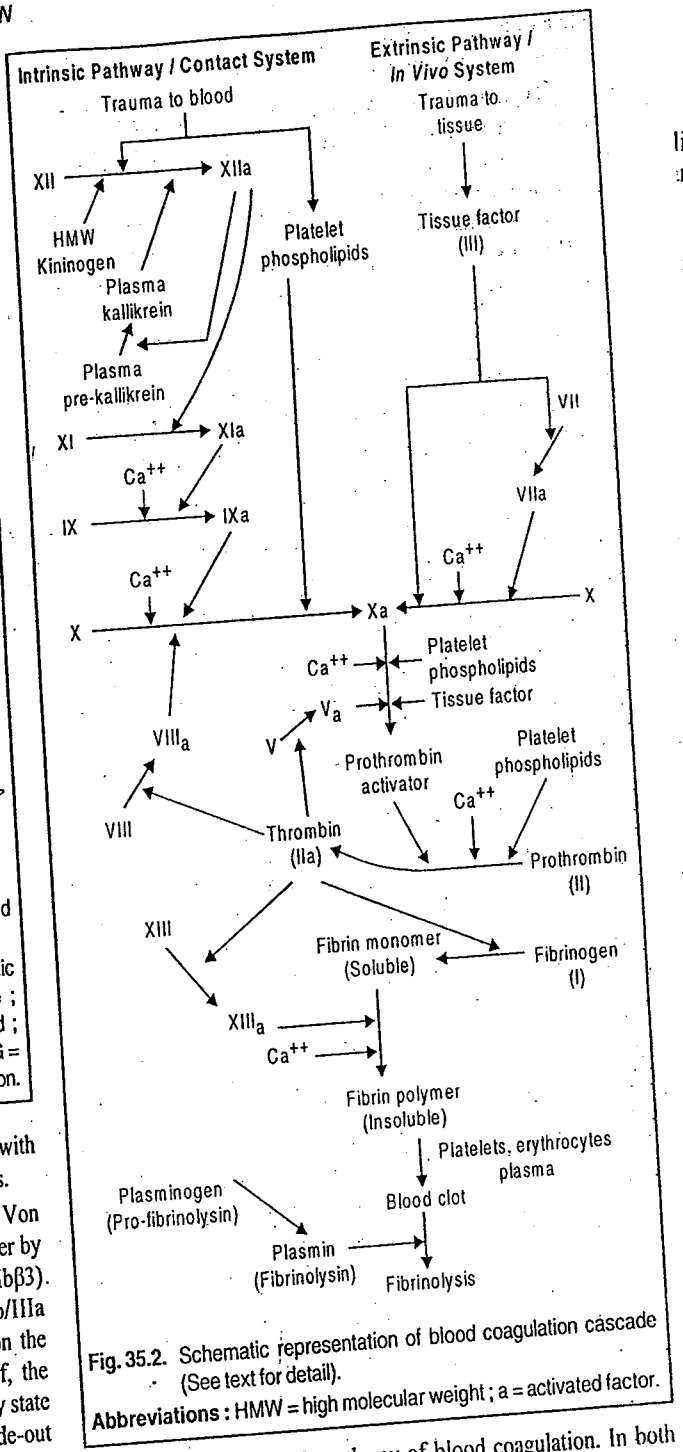


Fig. 35.2. Schematic representation of blood coagulation cascade (See text for detail).

Abbreviations : HMW = high molecular weight ; a = activated factor.

pathway and the intrinsic pathway of blood coagulation. In both pathways, certain coagulation proteins, generally called coagulation factors, participate in highly specific reactions. These coagulation factors are designated by Roman numeral and by the International Committee for Nomenclature of Blood Clotting Factors (Table 35.1). A lowercase '*a*' is appended to indicate an active form. These coagulation factors have been numbered from I through XIII. Factors III and VI are unassigned, as thromboplastin (earlier Factor III) actually turned out to consist of ten further factors, and accelerin was found to be activated Factor V. Although several new coagulation proteins or factors have been identified in recent years, but the assignment of numerals after the naming of Factor XIII has been stopped since 1963. The coagulation factors are generally serine proteases (enzymes), with exception of factors V, VIII and XIII. The Factors V and VIII are glycoproteins.

and Factor XIII is a transglutaminase. All coagulation proteins are produced in liver, except the factor VIII.

The extrinsic system (tissue factor pathway, rapid pathway) works more rapidly (10-15 seconds) and is more important *in vivo*. It is initiated by the activation of clotting Factor VII by a tissue factor thromboplastin. The intrinsic pathway (contact activation pathway, slow pathway) works slowly (1-6 minutes) and is triggered by the activation of clotting Factor XII, following its contact *in vitro* with glass or highly charged surface. Activation of Factor XII also requires high molecular weight (HMW) kininogen and is accelerated by pre-kallikrein. Both systems involve a cascade of enzymatic reactions which sequentially transform various inactive clotting factors (proenzymes) to their active (enzymatic forms), ultimately producing thrombin. Thrombin plays a key role in the coagulation, since it is responsible for generation of fibrin, a glycoprotein that forms the mesh-like matrix of blood clot (Figure 35.2). Thrombin also activates clotting Factor XIII (necessary for stabilising and cross linking fibrin molecules into an insoluble clot) and stimulates aggregation of platelets, production of arachidonic acid, and release of various mediators. Once thrombin forms and clotting begins, the proteolytic action of thrombin activates also Factor V (and Factor VIII) through the positive feedback effect and accelerates the entire process. Calcium and platelet phospholipids also play important role in steps in the coagulation scheme.

ANTIHAEMOSTASIS

Fibrinolysis

Fibrinolysis is the process wherein a fibrin clot, the product of coagulation, is broken down. In this process, a plasma protein called plasminogen or profibrinolysin is activated and converted to plasmin or fibrinolysin. Plasmin is a proteolytic enzyme that digests the fibrin fibres within 1-2 days of clot formation and causes lysis of the clot. It also causes destruction of many of the clotting factors (e.g. Factor V, Factor VIII, prothrombin, fibrinogen and Factor XII), thereby sometimes even causing hypocoagulability of the blood. Alpha$_2$-antiplasmin, a circulating glycoprotein, inactivates the free plasmin. The plasmin bound to fibrin is not inactivated by antiplasmin because of common binding site for both fibrin and antiplasmin. Tissue plasminogen activator (tPA), a serine protease found on endothelial cells of blood vessels, activates plasminogen and catalyzes the conversion of plasminogen to plasmin.

Inhibitory factors

Platelet activation and coagulation normally do not occur within an intact blood vessel because of presence of certain natural inhibitory factors. One of the most important inhibitor of coagulation system is a α_2-globulin antithrombin III, which neutralises not only thrombin but also other serine proteases in

Table 35.1: Blood coagulation factors.

Clotting Factor	Synonym(s)	Functional Activity	Deficiency Disease
Factor I	Fibrinogen	Substrate	Hypofibrinogenaemia, afibrinogenaemia.
Factor II	Prothrombin	Serine protease	Hypoprothrombinaemia, aprothrombinaemia.
Factor III	Tissue factor (Old name Tissue thromboplastin)	Activator	
Factor IV	Calcium	Cofactor	
Factor V	Proaccelerin; Labile factor; AC-globulin.	Cofactor	Parahaemophilia
Factor VI	Unnamed (Old name for Factor Va)		
Factor VII	Proconvertin; Stable factor; Serum prothrombin conversion accelerator; Autoprothrombin;.	Serine protease	Hypoproconvertinaemia
Factor VIII	Antiheamophilic factor A; Antihaemophilic globulin.	Cofactor	Haemophilia A, classic haemophilia.
Factor IX	Antihaemophilic factor B; plasma thromboplastin component; Christmas factor, Autothrombin II.	Serine protease	Haemophilia B, Christmas disease.
Factor X	Stuart factor; Stuart Prower factor.	Serine protease	
Factor XI	Plasma thromboplastin antecedent; Antihaemophilic factor C.	Serine protease	Haemophilia C
Factor XII	Hageman factor; Glass contact factor.	Serine protease	Hageman trait
Factor XIII	Fibrin stabilising factor; Fibrinase; Laki-Lorand factor.	Transaminase	
Von Willebrand factor (vWF)		Glycoprotein	von Willebrand disease.
Pre-kallikrein	Fletcher factor	Serine protease	
HMW Kininogen	Fitzgerald factor; Flaujeac-Williams factor.	Cofactor	

HMW = High molecular weight.

the enzymatic cascade including activated Factors X, IX, XI, and XII. Heparin sulphate proteoglycan synthesised by endothelial cells stimulate the activity of antithrombin. Antithrombin deficiency has been observed in cases of recurrent venous thrombosis and pulmonary embolism. Similar to antithrombin, another inhibitory factor called 'Protein C' degrades cofactors V_a and $VIII_a$ and greatly diminishes the rates of activation of prothrombin and Factor X. Similarly, tissues factor pathway inhibitor (TFPI), found in the lipoprotein fraction of plasma, inhibits Factor X_a and clotting Factor VII_a-tissue factor complex. Prostacyclin, synthesised by endothelial cells, inhibits platelet aggregation and helps in maintaining the flow of blood in intact blood vessel. Several other platelet aggregation inhibitors decrease the formation or the action of chemical signals which promote platelet aggregation.

Thus a check and balance system operates in body to maintain blood in a fluid state while in circulation and allows rapid haemostasis following injury. If the haemostasis or antihaemostasis mechanism is faulty or becomes exhausted, various coagulative disorders like haemophilia or disseminated intravascular coagulation may occur.

HAEMOSTATICS

Haemostatics (Haemostatic drugs) are agents which prevent or attenuate bleeding. These agents are primarily used to arrest bleeding or to control oozing from minute blood vessels. Haemostatics are useful also in some naturally occurring pathological conditions like haemophilia and in fibrinolytic states which may arise after surgery.

Classification

Depending on the route of administration and site of action, haemostatics have been broadly divided into 2 groups – topical haemostatics and systemic haemostatics.

I. Topical haemostatics
   1. Coagulants/Clotting factors
      e.g. thromboplastin, thrombin and fibrinogen.
   2. Occlusives/Artificial matrices
      e.g. fibrin foam, microfibrillar collagen, oxidised cellulose, gelatine sponge, calcium alginate, zeolites and chitosan.
   3. Vasoconstrictors
      e.g. epinephrine and norepinephrine.
   4. Styptics/Astringents
      e.g. ferric sulphate, silver nitrate, alum, tannic acid and zinc chloride.

II. Systemic haemostatics
   1. Coagulants
      a. Vitamin K and analogues
         e.g. phytomenadione, menatetrenone and menadione.
      b. Blood and blood components
         e.g. whole blood, fresh plasma, fresh frozen plasma, cryoprecipitate, platelet rich plasma, antihaemophilic factor, coagulation Factor IX (Recombinant), recombinant human factor VIIa and fibrinogen.
   2. Fibrinolytic inhibitors/Antifibrinolytic drugs
      e.g. aminocaproic acid, tranexamic acid and aprotinin.
   3. Miscellaneous drugs
      e.g. protamine sulphate, malonic and oxalic acids, adrenochrome monosemicarbazone, ethamsylate, carboprost, desmopressin and rutin.

I. TOPICAL HAEMOSTATICS

Topical haemostatics are applied directly on the denuded or bleeding surface and are useful in cases of superficial capillary bleeding. These agents usually provide an exogenous clotting factor or an artificial structural matrix that facilitates endogenous clotting. As these agents mainly facilitate endogenous coagulation mechanism, therefore an intact haemostatic mechanism is necessary for their efficacy. These agents are sometimes also called absorbable haemostatics because most of these drugs are absorbed/dissolved after some time of their topical application. The local haemostatics include a number of substances prepared from the blood itself.

1. Coagulants/Clotting Factors

Lyophilised concentrations of one or more clotting factors or blood proteins are available commercially for topical or local haemostatics.

Thromboplastin/Thrombokinase

Thromboplastin (Thrombokinase) is a plasma protein aiding blood coagulation through conversion of prothrombin to thrombin. Thromboplastin is produced naturally by platelets and damaged tissues when blood vessel is damaged, and it helps in clotting mechanism. Commercial thromboplastin is prepared more frequently as an extract of cattle brain in normal saline. It is used as local haemostatic in capillary bleeding as spray or direct application. It is also used for treating epistaxis and in surgery of nose, throat, bones and glandular tissues.

Thrombin

Thrombin, also called activated coagulation Factor II (II_a), is an enzyme that converts fibrinogen into fibrin and also stimulates Factors XIII, V and VIII. Commercially, bovine thrombin is available as a powder supplied in a dual pack with a suitable diluent. It is used as a local haemostatic in capillary bleeding, dental sockets, nose and throat surgery and reconstructive surgery. It is used as a powder or a solution in water or isotonic saline and may be used in conjunction with fibrinogen, fibrin foam or absorbable gelatine sponge. Thrombin is particularly very useful as an adhesive agent for skin grafts. Thrombin should not be injected parenterally, otherwise it may cause local ischemia and extensive intravascular clotting.

Fibrinogen/Human fibrinogen

Fibrinogen (Coagulation factor I) is a soluble plasma glycoprotein that is converted by thrombin into fibrin during blood coagulation. Human fibrinogen is available as a white powder or friable solid prepared from human plasma by precipitation with organic solvents and freeze-drying. It is used as 2% on denuded mucous membranes for capillary bleeding and as an adhesive in skin grafts. It is also used for restoring normal plasma fibrinogen levels in bleeding complications caused by acute afibrinogenaemia. Fibrinogen may be combined with thrombin solution to induce localised clotting.

2. Occlusives

Occlusives (Artificial matrices) are agents other than clotting factors which arrest bleeding or control oozing from minute

vessels by formation of an artificial clot or by providing an artificial matrix, which facilitates clotting. Unlike coagulants, these agents block the flow of blood mechanically and facilitate sealing of the vessels.

Fibrin foam

Fibrin is available commercially as a dry sterile fibrin sponge or foam prepared by the action of thrombin on human fibrinogen. It is marketed in the form of strips of a fine white sponge for covering or packing bleeding surfaces. It traps blood oozing from the surface area or a cavity and acts as a mechanical agent to promote blood clotting. It is usually left *in situ* from where it gets absorbed into the body. It may be combined with thrombin.

Microfibrillar collagen

Microfibrillar collagen haemostat (MCH) is a topical agent composed of resorbable microfibrillar collagen. When applied to a bleeding surface, the MCH patch attracts platelets and allows for the formation of a blood clot when it comes into contact with blood. The MCH is useful only in superficial bleeding. In heavy bleeding, blood vessel must be mechanically clamped and repaired.

Oxidised cellulose

Oxidised cellulose is a sterile surgical gauze prepared by the oxidation of cotton containing not less than 16% and not more than 22% of carboxyl group. It has a pronounced affinity for haemoglobin and produces an artificial clot. As a topical haemostatic, the cellulose is applied dry on bleeding surface, where it swells and forms a brown gelatinous mass. Oxidised cellulose is suitable for surface haemostasis only because it is not well absorbed and also interferes with healing of tissue. It is primarily used as a haemostatic in operations where ligation is not feasible (capillary or venous bleeding from small vessels). The oxidised cellulose is acid in reaction and must be neutralised with sodium bicarbonate, if used with thrombin solution. Oxidised cellulose is non-toxic and relatively non-irritating, but it interferes to some extent with wound healing. Oxidized cellulose should not be used in conjunction with thrombin as it inactivates the thrombin.

Gelatine sponge/Absorbable gelatine sponge

Gelatine sponge is a finely porous, white sponge-like material made from animal skin gelatine. It is available in various shapes and can absorb several (about 30) times its own weight of whole blood. It is moistened with saline or thrombin solution and left in bleeding area following closure of the operative wound. At the site of application, it liquefies within two to five days and is then completely absorbed within 4 to 6 weeks. While its mode of action is not fully understood, the effect of gelatine sponge appears to be more physical than the result of altering the blood clotting mechanism. It may be used in all types of surgery where control of capillary and venous bleeding is required. In addition to sponge, gelatine is also available in other forms like absorbable gelatine film and absorbable gelatine powder.

Calcium alginate

Calcium alginate is an absorbable haemostatic that is prepared by addition of aqueous calcium chloride to aqueous sodium alginate (salt of alginic acid). It is insoluble in water but soluble in sodium citrate solution. Dressings containing calcium alginate are available commercially for control of wound haemorrhage during surgical procedures. The dressing may be left at the bleeding site in the same manner as other absorbable dressings. Calcium alginate is also used as wound dressing to manage exudates in wounds, providing a moist environment for healing. Powdered alginate may be used to dust surgical gloves.

Zeolites

Zeolites are microporous, aluminosilicate minerals commonly used as commercial adsorbents. Zeolites are widely used in industry for water purification, as catalysts, and in nuclear reprocessing. Zeolites are also used nowadays as haemostatics to check bleeding from external injuries which cannot easily be controlled by direct pressure, elevation or a tourniquet. They work by adsorbing water from the blood, concentrating the clotting factors, activating platelets, and promoting steps in the coagulation cascade. Zeolites possess strong exothermic reactions and generate heat at the site of application, sometimes enough to cause burns. Newer versions of the zeolites have been recently prepared and are reported to generate less heat.

Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.) and cell walls of fungi. Chitosan has a number of commercial and biomedical uses. Chitosan haemostats, composing of chitosan and its salts, are topically used to check bleedings. Chitosan bonds with platelets and red blood cells to form a gel-like clot which seals the bleeding vessel. Unlike other haemostats, its action does not require the normal haemostatic pathway and therefore continues to function even when anticoagulants like heparin are present. Chitosan is used in some emergency haemostats which are designed to stop traumatic life-threatening bleeding. Its use is well established in many military and trauma units.

3. Vasoconstrictors

Epinephrine and Norepinephrine

Epinephrine and norepinephrine are catecholamines, which induce localised haemostasis by virtue of their vasoconstrictive effects. These agents are sometimes included in topical medications to decrease blood flow to the tissues. Epinephrine and norepinephrine may be applied intranasally in tampons as 1% to decrease epistaxis (gauzed soaked in epinephrine or norepinephrine solution is packed in the bleeding nostril).

4. Styptics/Astringents

Styptics (Stiptics) are agents which arrest bleeding by their astringent action. When applied locally, they cause precipitation of proteins of the blood and soft tissues and, therefore, seal the bleeding vessel. These agents do not penetrate into tissues and have only localised action. Styptics should not be used in high concentrations because they can be damaging to surrounding tissues. Examples of styptics include ferric sulphate, ferric chloride, silver nitrate, tannic acid, alum and zinc chloride. Tannic acid (20% in glycerine) is used in human medicine for bleeding gums, bleeding piles, etc. Minor arterial bleeding that occurs during docking of puppies may be controlled with strong solution of ferric chloride. Alum (hydrated potassium aluminium sulphate, potassium alum) in powder or crystal form, or in styptic pencils, is sometimes applied to cuts to prevent bleeding or treat infection.

II. SYSTEMIC HAEMOSTATICS

1. Coagulants

a. Vitamin K and Analogues

Vitamin K (Koagulation vitamin) is a fat-soluble vitamin that is essentially required as a cofactor for hepatic synthesis of blood coagulation Factors II (prothrombin), VII, IX and X. Deficiency of vitamin K results in deficiency of these clotting factors usually manifested by haemorrhage after minor trauma. Vitamin K is available in several forms including vitamin K_1 (phytonadione, phylloquinone or phytomenadione), vitamin K_2 (menaquinone or menatetrenone) and vitamin K_3 (menaphthone or menadione). Vitamin K is used as haemostatic only in instances of vitamin K deficiency (e.g. anticoagulant rodenticide toxicity and mouldy sweet clover poisoning). The therapeutic response to vitamin K is slow (requires about 24 hours for the hepatic synthesis of coagulation factors) and hence is not suitable for acute bleeding disorders. The pharmacology of vitamin K and analogues has been discussed in Chapter 54.

b. Blood and Blood Components

Fresh blood or blood components as haemostatic drugs are administered to animals which have a coagulation factor deficiency. These include fresh blood, fresh plasma, fresh frozen plasma, cryoprecipitate and platelet rich plasma.

Fresh blood/Fresh whole blood

Fresh whole blood is the whole blood that is administered within 6 hours of its collection. Fresh whole blood contains all coagulation proteins. It should be mixed gently before administration and must be used as early as possible after collection because some coagulation proteins (e.g. Factors V and VII) lose activity within few hours. The pharmacology of whole blood has been given in Chapter 36.

Fresh plasma

Fresh plasma is the plasma that is separated from erythrocytes and administered within 6 hours of collection. Fresh plasma contains electrolytes, albumin and some coagulation proteins. Plasma and related products should be administered cautiously because plasma contains majority of donor proteins (including antibodies). Transfusion reactions are not uncommon and, as with whole blood, may occur with the initial transfusion.

Dose

Dogs : 5 – 10 ml/kg, IV infusion (2-5 ml/kg/hour). It is repeated until bleeding is controlled.

Fresh frozen plasma

Fresh frozen plasma (FFP) is plasma that is frozen within 6 hours after collection, preferably at –40° to –80° C. Fresh frozen plasma contains vitamin K dependent factors (Factors II, VII, IX and X) and also coagulation Factor V. Frozen plasma (plasma separated from whole blood at any time after collection up to the expiration date of previously collected blood) does not contain Factors V and VII and is, therefore, less preferred than the fresh frozen plasma. Fresh frozen plasma can be stored at less than –40° C for 1 year or in a household freezer (–20° C) for 3 months, at which time it becomes frozen plasma. Fresh frozen plasma should be blood type-matched to ensure compatibility, as agglutination reactions are possible, though rare.

Fresh frozen plasma is used in patients in which clotting factors are deficient. It may also be used for reversal of warfarin effect and a source of antithrombin III in patients those are deficient of this inhibitor and are undergoing surgery or which require heparin for treatment of thrombosis. The risks of FFP include disease transmission, alloimmunization and excessive intravascular volume. Transfusion reactions may occur, as with whole blood.

Dose

Dogs : 10 ml/kg, IV infusion (2-5 ml/kg/hour). It is repeated until bleeding is controlled.

Cryoprecipitate

Cryoprecipitate, also called 'Cryoprecipitated Antihemophilic Factor', is a white foamy precipitate formed after centrifugation of partially thawed (slurry consistency) fresh frozen plasma that has been frozen for 6 months or less. Cryoprecipitate contains concentrated sources of coagulation factors VIII, von Willebrand factor, fibrinogen and fibronectin. Cryoprecipitate is indicated for haemophilia, von Willebrand disease, hypofibrinogenaemia and other specific conditions. Like fresh frozen plasma, compatibility testing is not strictly necessary, but should be done if possible.

Dose

Dogs : 1 unit/10 kg, slow IV infusion. It is repeated until bleeding is controlled.

Platelet-rich plasma

Platelet-rich plasma (PRP) is plasma containing high concentrations of platelets. The PRP also contains and releases (through degranulation) at least seven different growth factors (cytokines) which stimulate bone and soft tissue healing. Platelet-rich plasma requires centrifugation within 6 hours of collection, preferably at high speeds (1200 g), for a short time (2.5 minutes) at 20° C. After preparation, platelet products should be immediately used because these cannot be stored. Platelet-rich plasma is indicated for platelet deficiencies, including immune mediated thrombocytopenia. These may also be used to repair and accelerate healing of bones, joints, tendons, ligaments and certain other injured areas.

Dose

Dogs : 1 unit/10 kg, slow IV infusion.

Antihaemophilic factor/Factor VIII

Antihaemophilic factor (Factor VIII) is a concentrated antihaemophilic globulin prepared from pooled human plasma. It is indicated (along with human fibrinogen) in haemophilia and antihaemophilic globulin (AHG) deficiency. It is useful in controlling bleeding episodes in haemophilic patients, but its action is usually short lived (1-2 days). Ultra pure Factor VIII concentrates prepared by monoclonal antibody and DNA recombinant DNA technology is also available for use in human patients. It can be used in mild cases of haemophilia A and von Willebrand disease.

Coagulation factor IX (Recombinant)

Christmas factor or Factor IX is one of the serine proteases of the coagulation system. Deficiency of this protein causes hemophilia B. Recombinant factor IX is available commercially and is used in human medicine to treat Christmas disease. Coagulation Factor IX (Recombinant) has been associated with allergic reactions and is contraindicated in patients who have manifested life-threatening, immediate hypersensitivity reactions, including anaphylaxis, to the product or its components.

Recombinant human factor VIIa

Factor VII (formerly known as proconvertin) is one of the serine proteases involved in the coagulation cascade. Recombinant human factor VIIa (rhFVIIa) has been developed for use in uncontrollable bleeding in haemophilia patients (with Factor VIII or IX deficiency) who have developed inhibitors against replacement coagulation factor. Reports of increased risk of deep vein thrombosis, pulmonary embolism and myocardial infarction in association with the use of rhFVIIa have been cited in literature.

Fibrinogen

The fibrinogen fraction of human plasma is used clinically to control bleeding in acute afibrinogenaemia and in haemophilia and AHG deficiency states. It is administered by slow IV infusion.

2. Fibrinolytic Inhibitors/Afibrinolytics

Fibrinolytic inhibitors (Afibrinolytics) are substances which inhibit fibrinolysis, a process wherein a fibrin clot, the product of coagulation, is broken down. These drugs are used in patients with hyperfibrinolysis, a situation with markedly enhanced fibrinolytic activity, resulting in increased bleeding. Fibrinolytic inhibitors help to arrest bleeding and avoid the use of blood products such as fresh frozen plasma with its associated risks of infections or anaphylactic reactions.

Aminocaproic acid

Aminocaproic acid (ε-amino caproic acid, 6-aminohexanoic acid) is a synthetic drug chemically similar to amino acid lysine. It competitively blocks activation of plasminogen to plasmin and hence inhibits fibrinolysis. Aminocaproic acid is also thought to reduce the activation of some inflammatory enzymes which damage myelin in degenerative myelopathy. In human medicine, it is primarily used for treating hyperfibrinolysis-induced haemorrhage. It has also been used in the treatment of haemorrhage due to severe trauma, major surgery, leukaemia and hepatic cirrhosis. It is a specific antidote for overdose of a fibrinolytic agent. In veterinary medicine, aminocaproic acid has been used as a treatment of degenerative myelopathy in dogs (seen mostly in German Shepherds). It is available in both oral and parenteral formulations. Potential side effect of aminocaproic acid is nausea, vomiting, intravascular coagulation, hypotension and myopathy. Some dogs exhibit symptoms of GI irritation.

Dose

For adjunctive treatment of degenerative myelopathy

Dogs : 15 mg/kg, PO, 3 times daily.

Tranexamic acid

Tranexamic acid is a synthetic derivative of the amino acid lysine and an analogue of aminocaproic acid. It acts like aminocaproic acid and competitively inhibits the activation of plasminogen to plasmin, a molecule responsible for the degradation of fibrin. It is about 8 times more potent than the aminocaproic acid and may also enhance the tendency towards intravascular coagulation. Its pharmacological properties and side/adverse effects profile is similar to those of aminocaproic acid. Like aminocaproic acid, it is mainly used in human beings in hyperplasminaemic/hyperfibrinolytic states. In human medicine, tranexamic acid is used as first line non-hormonal treatment of dysfunctional uterine bleeding and heavy bleeding associated with uterine fibroids. It is also used in orthopaedic and cardiac surgery to prevent excessive blood loss.

Aprotinin

Aprotinin is a 6.5 kDa protein obtained from bovine lung. It inhibits some proteolytic enzymes including plasmin and kallikrein (see also Chapter 28). It has been used primarily in human medicine for the hyperplasminaemia produced by fibrinolytic agents and to reduce excessive bleeding during many types of surgery, especially involving cardiac surgery. The important adverse effects of aprotinin include hypersensitivity reactions and venous or arterial thrombosis. Presently all aprotinin preparations have been withdrawn from market due to reports of its increased risk of complications or death.

3. Miscellaneous Drugs

Protamine sulphate

Protamine is a low molecular weight cationic protein that was originally isolated from the sperm of salmon and other species of fish but is now produced primarily through recombinant biotechnology. It is strongly basic in nature because it contains high arginine contents in its structure. Protamine acts as an antagonist of heparin. In body, the positively charged (basic) protamine interacts with the negatively charged (acidic) heparin to form a stable complex, which has no anticoagulant activity. The complex of heparin and protamine is then removed and broken down by the reticuloendothelial system. Protamine sulphate itself has weak anticoagulant activity when it is given in the absence of heparin, because the basic protein has a tendency to interact with platelets and fibrinogen.

Protamine sulphate is used clinically as an antagonist of heparin. It is useful in all species for the treatment of heparin overdosage when significant bleeding occurs. Protamine sulphate should not be used in individuals who are allergic to fish, as it can cause significant histamine release resulting in hypotension, bradycardia, bronchoconstriction and dyspnoea, and also causes pulmonary hypertension. Slow infusion minimizes these side effects. It has also been used for Bracken Fern toxicity in ruminants. Protamine should not be used to check bleeding other than that caused by heparin because it may aggravate bleeding due to its inherent anticoagulant activity.

Dose

For heparin overdosage

All species : 1 mg protamine for each 100 units (1 mg) of heparin to be inactivated, slow IV or infusion. Dose is reduced by half for every 30 minutes which have lapsed after administration of heparin. No more than 50 mg should be given in a 10-minute period.

Oxalic acid and Malonic acid

A mixture of oxalic acid and malonic acid in equal concentrations (5% each) has been used clinically to check haemorrhage in domestic animals. Although the precise mechanism of haemostatic effect of drugs is not known, the acids probably cause rupture of platelets and liberate coagulation factors.

Dose

Small animals : 0.5 – 3 ml (total dose), IV or IM.

Large animals : 5 – 20 ml (total dose), IV or IM.

Adrenochrome monosemicarbazone

Adrenochrome is an oxidation product of epinephrine. Adrenochrome monosemicarbazone (Carbazochrome) is used for

systemic control of capillary bleeding associated with increased capillary permeability. It is believed to reduce capillary fragility and control oozing from raw surfaces. It is used both orally and parenterally, and is often given in combination with vitamin K for variety of bleeding disorders like epistaxis, haematuria and secondary haemorrhage from wounds. Its efficacy, however, is questionable.

Ethamsylate

Ethamsylate (Diethyl ammonium 2,5-dihydroxybenzene sulphonate) is a systemic haemostatic agent that reduces capillary bleeding. It is believed to work by maintaining capillary integrity, probably by promoting polymerisation of mucopolysaccharide in vessel walls and platelet adhesion. It also inhibits biosynthesis and action of those prostaglandins which cause platelet disaggregation, vasodilatation and increased capillary permeability. Following systemic administration, the mean bleeding time is significantly reduced without any effect on cell counts, fibrinolysis, prothrombin time or clotting time. The drug is excreted unchanged, mainly in the urine. Ethamsylate is used mainly for the treatment of capillary haemorrhage, haematemesis, epistaxis, menorrhagia and post-partum haemorrhage.

Carboprost

Carboprost is a synthetic analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Carboprost induces contraction of uterine muscles and has significant oxytocic action. It is also used as haemostatic especially for the treatment of post-partum haemorrhage not controlled by oxytocin or ergometrine. The pharmacology of prostaglandin $F_{2\alpha}$ and analogues have been discussed in Chapter 29.

Desmopressin

Desmopressin, a synthetic analogue of vasopressin, provides haemostatic action in animals suffering from von Willebrand disease. It causes release of preformed von Willebrand factor (with subsequent increase in factor VIII) from endothelial cells and macrophages and transiently elevates its serum concentration. The increase in the serum levels of von Willebrand factor is sufficient to provide improved coagulation activity in animals suffering from deficiency of the factor. Desmopressin is also used in treatment of mild haemophilia A (factor VIII deficiency) and thrombocytopenia. However it is not effective in the treatment of haemophilia B (factor IX deficiency) or severe haemophilia A. Repetitive administration of desmopressin results in depletion of storage pools of the factor and loss of haemostatic effect. The pharmacology of desmopressin is given in Chapter 49.

Rutin

Rutin, also called rutoside, is a plant glycoside that is used occasionally to reduce capillary bleeding. It strengthens the capillaries and can reduce the symptoms of haemophilia. It is used mainly in human medicine in combination with vitamin C and vitamin K. Rutin is occasionally used in the management of chylothorax (a type of pleural effusion, resulting from lymphatic fluid (chyle) accumulating in the pleural cavity) in dogs and cats.

ANTIHAEMOSTATICS

Antihaemostatics (Antihaemostatic drugs) are agents which reduce or prevent haemostasis or blood clotting. These drugs are used clinically to limit formation of thrombi in a variety of disease processes which disrupt normal haemostatic mechanism and lead to excessive coagulation and thrombosis. Accordingly they are also called antithrombotic drugs.

Classification

Pharmacological agents which inhibit haemostatic mechanism are categorised broadly into three groups: anticoagulants, thrombolytics and antithrombotics.

I. Anticoagulants
  A. *In vitro* anticoagulants
    1. Anticoagulants used for laboratory purposes
      e.g. oxalates, sodium fluoride and ethylenediamine tetra acetic acid.
    2. Anticoagulants used for blood transfusion and laboratory purposes
      e.g. sodium citrate, acid citrate dextrose and heparin.
  B. *In vivo* anticoagulants/Systemic anticoagulants
    1. Heparin and related substances
    a. Heparin and derivatives
      e.g. heparin and low molecular weight heparins.
    b. Heparinoids
      e.g. heparan sulphate, danaparoid, dextran sulphate, dermatan sulphate and sulodexide.
    c. Oligosaccharides
      e.g. fondaparinux and idraparinux.
    2. Inhibitors of vitamin K
    a. Coumarin derivatives/Coumarins
      e.g. warfarin, dicoumarol, acenocoumarol, ethylbiscoumacetate, phenprocoumon, coumatetralyl and cyclocoumarol.
    b. Indanedione derivatives/Indanediones
      e.g. anisindione, phenindione, diphenadione and clorindione.
    3. Direct inhibitors of coagulation factors
    a. Direct inhibitors of Factor X_a
    i. Xabans
      e.g. rivaroxaban, apixaban, betrixaban, edoxaban and otamixaban.
    b. Direct inhibitors of Factor II
    i. Hirudin and congeners
      e.g. hirudin, bivalirudin, desirudin and lepirudin.
    ii. Miscellaneous direct thrombin inhibitors
      e.g. argatroban, dabigatran and ximelagatran.
    4. Miscellaneous anticoagulants
      e.g. defibrotide, drotrecogin alfa, antithrombin III (Human) and ramatroban.

II. Thrombolytics/Fibrinolytics
  1. Plasminogen activators
    e.g. tissue plasminogen activator (alteplase, reteplase tenecteplase and monteplase), streptokinase, urokinase, streptodornase, anistreplase and saruplase.
  2. Others drugs
    e.g. ancrod, fibrinolysin and brinase

III. Antithrombotics/Antiplatelet drugs/Antiaggregants
  1. Cyclooxygenase inhibitors

e.g. aspirin, aloxiprin, carbasalate calcium, indobufen and triflusal.

2. Adenosine diphosphate (ADP) receptor inhibitors
   a. Thienopyridines
      e.g. clopidogrel, prasugrel and ticlopidine.
   b. other drugs
      e.g. ticagrelor.
3. Glycoprotein IIb/IIIa inhibitors
   e.g. abciximab, eptifibatide and tirofiban.
4. Thromboxane inhibitors
   e.g. dipyridamole, picotamide, dazoxiben and terutroban.
5. Miscellaneous drugs
   e.g. cilostazol, sulfinpyrazone, cloricromen, ditazole and timolol.

I. ANTICOAGULANTS

Anticoagulants (Anticoagulant drugs) are agents which inhibit coagulation of blood. These drugs are used either *in vitro* to prevent clotting of blood for transfusion, diagnostic or analytical use, or *in vivo* to prevent development and enlargement of thrombi.

A. *In vitro* anticoagulants

1. Anticoagulants Used For Laboratory Purposes

Certain anticoagulants are used mainly for laboratory diagnostic and analytical purposes. They share a common mode of action in that they render ionic calcium (Factor IV) unavailable preventing the coagulation proteins from using it. These agents are not suitable for blood transfusion or *in vivo* conditions due to their harmful effects.

Oxalates

Oxalates are available as sodium, potassium, ammonium and lithium salts. Sodium and potassium oxalates are more commonly used than other oxalate salts. Oxalates combine with blood calcium and form insoluble calcium oxalate. This renders calcium unavailable for blood coagulation. The oxalates have a limitation that they cause distortion of cells and clumping of platelets. All oxalates salts are toxic, so they must not be used for blood transfusion or administered *in vivo* for treatment of haemostatic disorders.

For laboratory purposes

Sodium oxalate : 2 mg per ml of blood.

Sodium fluoride

Sodium fluoride is an inorganic chemical compound with the formula NaF. Sodium fluoride combines with calcium and forms a calcium component, which is poorly dissociated. It is an excellent anticoagulant for blood glucose studies because it interferes with some enzymes involved in glycolysis. In addition to anticoagulant action, sodium fluoride acts as a preservative. It can even be added to blood having other anticoagulants.

For glucose preservation

Sodium fluoride: 2.5 mg per ml of blood.

Ethylenediamine tetra acetic acid

Ethylenediamine tetra acetic acid (Edetate), commonly abbreviated as EDTA, is a laboratory reagent that is widely used as an anticoagulant. Sodium and potassium salts of EDTA are mostly used to chelate the blood calcium and prevent clotting. In this form, calcium is still present in soluble form, but is no longer a free ion. The disodium salt of EDTA is more soluble than the dipotassium salt and, therefore, is preferred for laboratory purposes. Ethylenediamine tetra acetic acid is preferred anticoagulant for thrombocyte count and other haematological studies because cellular details are preserved well. Excess of EDTA causes shrinkage of erythrocytes causing error in erythrocyte indices. Ethylenediamine tetra acetic acid is not useful for blood transfusion.

EDTA (disodium : 0.1 – 0.2 mg per ml of blood.
or dipotassium salt) : 0.01 ml of 2% EDTA solution per ml of blood.

2. Anticoagulants Used For Blood Transfusion and Laboratory Purposes

Some anticoagulants are used to keep blood in the fluid state for blood and blood plasma transfusions. They are also used for various laboratory diagnostic and analytical purposes.

Sodium citrate

Sodium citrate (Trisodium citrate) is a laboratory reagent that is mainly used to prevent coagulation of blood collected for transfusion purposes. Like other laboratory anticoagulants, it chelates the blood calcium and makes it unavailable for blood coagulation. Sodium citrate, unlike other calcium chelators, is safe to the recipient because it is rapidly metabolised (citrate radical enters the Krebs cycle) and excreted from the body. Sodium citrate contains no preservative or energy sources. Collected blood can be refrigerated for up to 35 days. Sodium citrate is also used occasionally for laboratory diagnostic and analytical work, especially for the study of platelet morphology and functions. The disadvantages include shrinkage of cells and interference with many chemical tests. Excess of sodium citrate *in vivo* may cause tetany (hypocalcaemic tetany) and impairment of muscular work.

For blood transfusion

1 ml of 3.5% solution of sodium citrate per 9 ml of blood (1 in 10).

For laboratory purposes

2.5 mg of sodium citrate per ml of blood.

0.01 ml of 25% solution of sodium citrate per ml of blood.

Acid citrate dextrose

Acid citrate dextrose (ACD) is a solution of citric acid, sodium citrate and dextrose in water. It is prepared by adding 2.5 g sodium citrate, 0.8 g citric acid and 2.5 g dextrose in distilled water to make 100 ml. Acid citrate dextrose is also commercially available in pre-packed quantities which can be dissolved in sterile water, sterilised and used to collect blood. It is used mainly for blood transfusion. It preserves erythrocytes better than sodium citrate alone and, therefore, is preferred for blood transfusion. Acid citrate dextrose preserves the blood for transfusion for up to 3 to 4 weeks. It is also used during procedures such as plasmapheresis instead of heparin.

For blood transfusion

15 ml of ACD per 100 ml of blood.

Heparin

Heparin is used both *in vitro* and *in vivo* to prevent or retard coagulation of blood. Its use *in vitro* conditions is limited mainly for some biochemical and haematocrit studies. It is also used to collect small quantities of blood (about 50 ml) for transfusion in cats or paediatric patients. Blood collected with heparin cannot

be stored because heparin contains no preservative and gets inactivated within 24 to 48 hours. Heparin also activates platelets rendering them non-functional, an undesirable effect if host is deficient in platelets. The pharmacology of heparin has been described in the present chapter under the topic *in vivo* anticoagulants.

For laboratory purposes

8 – 10 Units of heparin per ml of blood.

For blood transfusion purposes

4 – 6 Units of heparin per ml of blood.

B. Invivo Anticoagulants/Systemic Anticoagulants

1. Heparin and Related Substances

a. Heparin and Derivatives

Heparin

Heparin (Unfractionated heparin, UFH) is an injectable rapid acting anticoagulant which is often used therapeutically for preventing enlargement of thrombi. Heparin was so named because of its discovery in high concentrations in the liver. Pharmaceutical-grade heparin is derived from mucosal tissues of slaughtered meat animals such as porcine intestinal mucosa and bovine lung, and is available as either sodium or potassium salt. The sodium salt is usually preferred for *in vivo* use. Heparin also occurs physiologically mainly in mast cells complexed with histamine where its exact function in unknown. Heparin is also stored in basophiles and, to a lesser extent, in vascular endothelium.

Chemistry

Heparin is not a single substance but is a family of linear, polyanionic, sulphated glycosaminoglycans (mucopolysaccharides). Each heparin molecule is composed of repeating units of D-glucosamine and D-glucuronic acid, or D-glucosamine and L-iduronic acid. Some glucosamine residues are N-acetylated. All heparin molecules contain a unique pentasaccharide sequence (Figure 35.3), which binds to antithrombin III and serves as active site of the molecules. The number of active sites is variable. The chain length and proportion of the two sulphated disaccharide units vary among heparin molecules which have molecular weight from 3,000 to 30,000 Da (mean 15,000 Da).

Heparin contains electronegative charges and has the highest negative charge density of any known biological molecule. The electronegativity and acidic character of heparin is due to presence of sulphate and also carboxylic acid groups. Therefore, the anticoagulant action of heparin is lost by contact with positively charged basic substance protamine. One unit of heparin (the "Howell Unit") is an amount approximately equivalent to 0.002 mg of pure heparin, which is the quantity required to keep 1 mL of cat's blood fluid for 24 hours at 0°C.

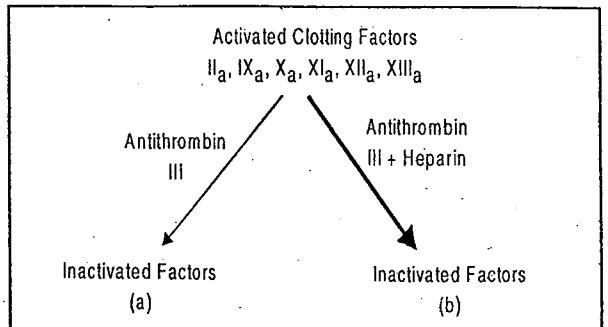


Fig. 35.4. Mechanism of action of heparin.
(a) In absence of heparin, inactivation of clotting factors by antithrombin III occurs slowly (normal arrow).
(b) In presence of heparin, inactivation of clotting factors by antithrombin III occurs very rapidly (bold arrow).

Mechanism of action

The mechanism of action of heparin as an anticoagulant is indirect. It facilitates the anticoagulant action of endogenous inhibitory factors (endogenous serine proteinase inhibitors), especially the antithrombin III (AT III) and heparin cofactor II (HC II). By binding to the endogenous coagulant inhibitory factors, heparin accelerates (about 10,000 fold) the velocity, but not magnitude, of interaction between the coagulant inhibitory factors and clotting factors. The AT is most efficient at inactivating thrombin (Factor II_a) and Factor X_a. Other factors neutralised by AT include activated factors IX, XI, XII, XIII and kallikrein (Figure 35.4). A specific five saccharide sequence (called AT-binding pentasaccharide), present within up to one third of unfractionated heparin chains, is essentially required to bind to AT. This AT-binding pentasaccharide sequence is also present in low molecular weight (LMW) heparins.

For inhibition of activated thrombin, it is necessary for heparin to bind to both the activated thrombin and antithrombin III together. The formation of a ternary complex between AT, activated thrombin and heparin requires at least 18 saccharide units, besides containing the specific AT-binding pentasaccharide sequence, which are present only in unfractionated high molecular weight heparin. In contrast, inhibition of the factor X_a requires only the AT-binding pentasaccharide sequence, which is present in both UF heparin and LMW heparins. The high molecular weight unfractionated

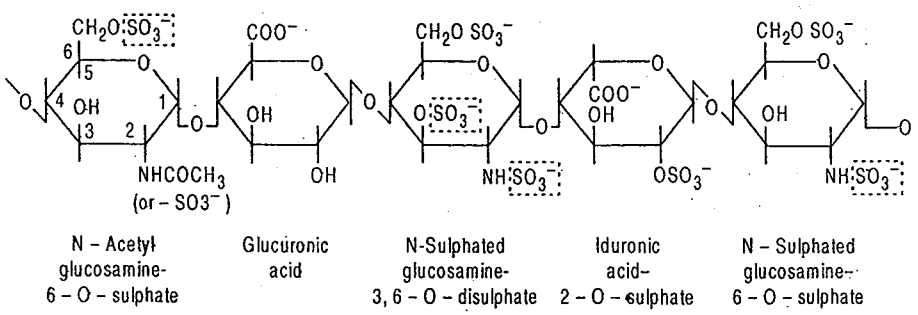


Fig. 35.3. The antithrombin III - binding pentasaccharide sequence of heparin. Sulphate groups required for binding to antithrombin III are indicated in dotted boxes ; other sulphate groups may increase AT III binding but are not essential for it.

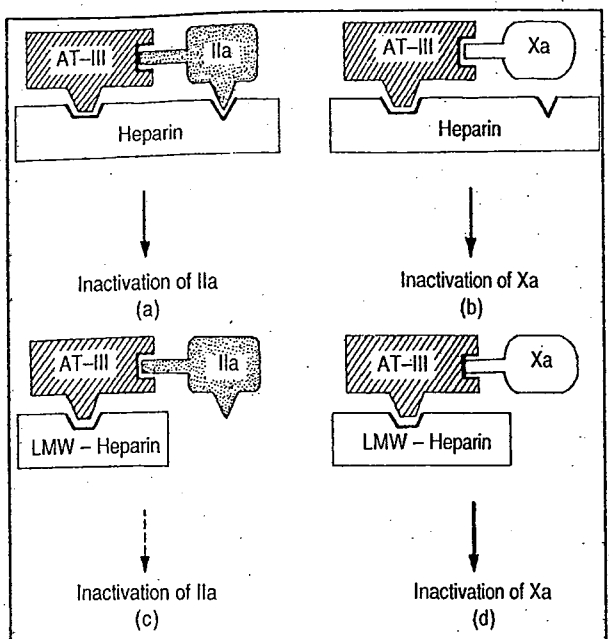


Fig. 35.5. Schematic representation of mechanism of action of heparin and low-molecular weight heparins. To enhance the rate of inactivation of thrombin (IIa) by antithrombin III, heparin needs interaction with AT-III (See also text).

(a) Heparin inactivates thrombin (IIa) by binding to both AT III and IIa simultaneously.

(b) Heparin inactivates Factor Xa by binding only to AT III. Inactivation of Xa does not require direct interaction with heparin.

(c) LMW - Heparin cannot inactivate IIa because it has no binding site for IIa.

(d) LMW - Heparin inactivates Factor Xa by binding to AT-III.

Abbreviations : AT III = antithrombin III ; IIa = activated Factor II (thrombin); Xa = activated Factor X ; LMW heparin = low molecular weight heparin; Bold arrow = very rapid action ; broken arrow = no/ negligible action.

heparin has comparatively lower affinity for AT III than the low molecular weight heparins, but it is more effective anticoagulant because its high molecular weight and large structure facilitates binding of both AT III and thrombin simultaneously (Figure 35.5a), in addition to binding to activated Factor X (Figure 35.5b). Unlike HMW heparins, LMW heparin fractions have a very high affinity for AT III, but they are more selective against Factor X_a than the activated thrombin (Factor II_a) because their smaller size possesses binding sites for only Factor X_a (Figure 35.5d) and not for activated thrombin (Figure 35.5c).

Other actions of heparin include antiplatelet effect due to its high affinity for platelet Factor IV. Most heparin preparations inhibit platelet aggregation and adhesion. Heparin also binds to vascular endothelium and alters vascular endothelium permeability. In addition, heparin interacts with many proteins (e.g. pro-inflammatory chemokines, growth factor and leucocyte proteins) probably by electrostatic forces between the polyionic groups of the glycosaminoglycan and the cationic groups of proteins.

Pharmacological effects

Anticoagulation effects: Intravenous administration of heparin produces anticoagulation within minutes. Low concentration of heparin, when combined with antithrombin III, inactivates Factor X_a and prevents conversion of prothrombin to thrombin. In high concentrations, it inactivates thrombin and blocks conversion of fibrinogen to fibrin, and neutralises activated Factors IX, X, XI, and XII. By inhibiting Factor XIII, heparin also prevents formation of stable fibrin clots. It does not lyse clots, but can prevent the growth of existing clots. Heparin in high doses also inhibits platelet aggregation and prolongs the bleeding time.

Other effects: Heparin has several other effects not related to its anticoagulation activity. In human beings, heparin reduces lipemia following a fatty meal by causing redistribution of plasma lipoproteins fractions. It releases the enzyme lipoprotein lipase that hydrolyses triglycerides to free fatty acids which then enter tissues. Heparin can inhibit or modulate selected targets of an inflammatory response, including neutrophils and T cells. Heparin appears to decrease leucocytes adherence to vascular endothelium and facilitates migration along a chemoattractant gradient to sites of inflammation. Heparin is also reported to block mast cell degranulation by altering calcium release. Heparin also appears to influence tumour cell metastasis. It inhibits aldosterone secretion in human patients causing natriuresis.

Pharmacokinetics

Heparin is not absorbed from the GI tract because of its large size and polarity, so it must be given parenterally to be effective. Administration of heparin is done by continuous infusion, intermittent IV injection, or deep subcutaneous injection. Intramuscular injections are avoided because they result in haematoma formation. Anticoagulant activity begins immediately after direct IV bolus injection, but it may take up to 1 hour after deep SC injection. Heparin is extensively protein bound, primarily to fibrinogen, low-density lipoproteins and globulins, which may decrease its bioavailability. It does not cross blood-brain barrier, placenta or enter milk.

Heparin is metabolised by heparinase in the liver and by reticuloendothelial cells. Metabolites of heparinase activity are excreted in urine (heparin released from mast cells is degraded by tissue macrophages; it is not physiologically circulating anticoagulant). The elimination half-life of heparin appears to be molecular weight dependent. The high molecular weight heparins show dose-dependent half-life thought to be due both to their high affinity for tissue proteins and saturable renal excretion. The half-life of unfractionated heparin is about 2 hours in humans which may increase to 5 hours with increase in dose. In contrast to HMW heparins, the LMW heparins have a longer half-life that is independent of dose.

Side effects/Adverse effects

Haemorrhage and thrombocytopenia are major complication of heparin therapy, more frequently occurring with overdosage. Haemorrhage is less likely to occur with low dosage and constant intravenous infusion in comparison with intermittent intravenous administration. Because heparin is derived from bovine or porcine tissues, hypersensitivity reactions are also possible. In human patients, long-term therapy may lead to osteoporosis and diminished renal function. A smaller number of human patients develop skin necrosis.

Treatment: Heparin overdosage can be treated by administration of protamine sulphate, which is a specific antidote to heparin.

Contraindications and precautions

Heparin is contraindicated for the bleeding patient and for disseminated intravascular coagulopathy (DIC), unless replacement blood or plasma therapy is given. Heparin should not be used in

Table 35.2: Comparative features of unfractionated heparin and LMW heparins.

Features	Heparin (Unfractionated)	LMW heparin
Average Mol. weight	15 kDa	4.5 kDa
Inhibition of thrombin	High	Low
Pharmacokinetic profile	Less predictable	More predictable
Elimination half-life	Short	Long
Risk of bleeding and thrombocytopenia	High	Low
Route of administration	IV infusion (Mainly)	SC (Mainly)
Antagonistic effect of protamine	Significant	Insignificant
Monitoring of activated partial thromboplastin time (aPTT)	Required	Not required

patients hypersensitive to it. Intramuscular administration of heparin is not advised as this may lead to haematoma formation. Haematoma, pain and irritation may also occur after deep SC dosing. Although heparin does not cross placenta, it should be used cautiously and only when necessary in pregnant animals. Blood coagulation time (e.g. activated partial thromboplastin time) should be monitored during heparin therapy.

Drug interactions

Heparin shows interactions with many drugs hence it should not be mixed with any other drug. Aspirin, phenylbutazone, warfarin, etc. may aggravate the bleeding, if used during heparin therapy. Heparin may antagonise actions of corticosteroids, insulin or ACTH. It also interferes with thyroid hormones causing decreases in both thyronine and thyroxine. Antihistamines, digoxin and tetracycline may partially counteract the actions of heparin.

Clinical uses

Heparin is one of the oldest drugs currently still in widespread clinical uses. In veterinary practice, heparin in used mainly for the prevention or treatment of venous (red thrombus) or pulmonary embolism and arterial fibrillation with embolisation (e.g. feline cardiomyopathy). Heparin and other anticoagulants are generally ineffective in the treatment of arterial (white) thrombi. Heparin is the drug of choice when rapid anticoagulant activity is necessary in acute thrombosis. Heparin is often used in conjunction with blood and plasma for the treatment of disseminated intravascular coagulopathy (DIC). Heparin is also used as an anticoagulant for diagnostic use and blood transfusion. Heparin has been used to clear hyperlipidaemia.

Dose

Dogs	:	40 – 80 Units/kg, SC, 3 times daily.
	:	100 – 200 Units/kg, IV (initial dose), followed by 50 units/kg, every 3 hours.
Cats	:	200 units/kg, SC, 3 times daily.
Horses	:	80 – 100 Units/kg, IV, after 4 to 6 hours for high grade DIC.
	:	25 – 40 Units/kg, SC, 2 to 3 times daily for low grade DIC.

Low Molecular Weight Heparins

Low molecular weight (LMW) heparins are short chains of polysaccharide that have been isolated from standard heparin by different techniques (e.g. gel filtration chromatography and differential precipitation with ethanol). These heparins essentially contain the AT-binding pentasaccharide sequence of heparin and have molecular weight ranging from 1000 to 10,000 Da with mean weight of 4,500 Da. Low molecular weight heparins differ from standard heparin in both pharmacokinetic properties and mechanism of action. They have very high affinity for antithrombin III (AT III), but act only by inducing confirmations changes in AT III and not by bringing together AT III and thrombin (Figure 35.5). Therefore they selectively inhibit Factor X_a with little effect on thrombin (Factor II). As a result, LMW heparins have little effect on activated partial thromboplastin time (aPTT) and whole blood clotting time than unfractionated heparin. They also have less antiplatelet action. Advantages of LMW heparins when compared with unfractionated heparin include better absorption after SC injection, prolonged elimination half-life, lower incidence of haemorrhagic complications and specificity of action (see also Table 35.2)

Some LMW heparins have been studied in humans and are available commercially for prophylaxis and treatment of venous thrombosis. They are also used to maintain patency of canulae and shunts in dialysis patients. Because LMW heparins have more predictable pharmacokinetics and anticoagulant effects, they are recommended over unfractionated heparin for patients with massive pulmonary embolism. Selected LMW heparins include ardeparin (1100 Da), bemiparin (3600 Da), certoparin, enoxaparin (4500 Da), dalteparin (5000 Da), reviparin (3900 Da), nadroparin (4500 Da), parnaparin (5000 Da) and tinzaparin (6500 Da). Enoxaparin fragment is considered as the prototype LMW heparin.

b. Heparinoids

Heparinoids are non-heparin naturally occurring and synthetic sulphated glycosaminoglycans (mucopolysaccharides), which possess heparin-like anticoagulant action. Heparinoid preparations have been used for a wide range of applications including as anticoagulant and anti-inflammatory agents.

Heparan sulphate

Heparan sulphate is a heparin like linear polysaccharide found on cell surface and extracellular matrix in many tissues. It is closely related to heparin and is synthesised from the same repeating disaccharide precursor. However, it contains higher proportions of glucuronic acid and N-acetyl glucosamine and fewer sulphate groups than the heparin. It functions like heparin by binding to antithrombin, but is less potent anticoagulant than heparin. Heparan sulphate also produces an anticoagulant effect *in vitro*.

Danaparoid

Danaparoid sodium (Orgaran) is a mixture of low molecular weight sulphated glycosaminoglycuronans derived from porcine intestinal mucosa. It consists of heparan sulphate (about 80%), dermatan sulphate (8 to 16%) and chondroitin sulphate (<8.5%).

but is devoid of heparin or heparin fragments. Compared to heparin, danaparoid has a much higher anti-factor X_a/anti-II_a ratio (more than 20:1). Danaparoid exerts a stronger catalytic effect on the inactivation of factor X_a than on the inactivation of thrombin. The anti-X_a activity is mediated by AT-III and the antithrombin activity is mediated by both AT-III and heparin cofactor II. Danaparoid has been shown to possess antithrombotic action and inhibits thrombus formation with approximately the same potency as heparin. It has minimal or no effect on the platelet functions.

Danaparoid is used in the prevention of deep vein thrombosis (DVT) following orthopaedic, major abdominal and thoracic surgery. Patients with a positive diagnosis of non-haemorrhagic stroke may also be treated with danaparoid. It has also been used as a substitute for heparin in human patients with heparin-induced thrombocytopenia. Danaparoid is intended primarily for SC use, but can also be administered as an IV bolus. Danaparoid possesses a risk of systemic bleeding as seen with all antithrombotic drugs. Its use is contraindicated in conditions like severe haemorrhagic diathesis, uncontrollable active bleeding state, hypersensitivity to danaparoid or any of its components and gastric or duodenal ulcer. Danaparoid should not be administered by IM route. Danaparoid should be used with caution in patients with severely impaired renal function because the main route of elimination is via the kidney. Determination of anti-factor X_a levels in plasma is the only method available for monitoring danaparoid activity.

Dextran sulphate

Dextran sulphate is a polyanionic derivative of dextran, a branched glucan (polysaccharide made of many glucose molecules) composed of chains of varying lengths. It is supplied as the sodium salt which is a white to off-white powder freely soluble in water and salt solutions to form a stable, clear solution. The anticoagulant effects of dextran sulphate are similar to those of heparin, but it is 7 times less potent than heparin. Dextran sulphate of various molecular weights are used as ingredients of creams and ointments for treating thrombophlebitis and for cosmetic applications. Dextran Sulphate has also found many applications in the fields of molecular biology for accelerating the hybridization rates of DNA fragments.

Dermatan sulphate

Dermatan sulphate is a glycosaminoglycan (mucopolysaccharide) found mostly in skin, but also in blood vessels, tendons and lungs. The two repeating sugar units in dermatan sulphate are iduronic acid (IdUA) and n-acetylgalactosamine (GalNAc). Dermatan sulphate has important physiological and patho-physiological roles in blood coagulation, cardiovascular disease, carcinogenesis, infection, wound repair and fibrosis. As an anticoagulant, it is sometimes injected along with heparin and a few other chemicals in the same family.

Sulodexide

Sulodexide is a highly purified mixture of glycosaminoglycans composed of low molecular weight heparin (80%) and dermatan sulfate (20%). Due to the presence of both glycosaminoglycan fractions, sulodexide potentiates the antiprotease activities of both antithrombin III and heparin cofactor II simultaneously. The pharmacological effects of sulodexide differ substantially from other glycosaminoglycans and are mainly characterized by a prolonged half-life and reduced effect on global coagulation and bleeding parameters. It is also efficiently absorbed after oral administration, compared to the unfractionated heparin. Sulodexide is mainly used for the prophylaxis and treatment of thromboembolic diseases.

c. Oligosaccharides

Fondaparinux

Fondaparinux is a synthetic pentasaccharide, possessing chemical structure and anticoagulant action similar to low molecular weight heparins. Apart from the O-methyl group at the reducing end of the molecule, the identity and sequence of the five monomeric sugar units contained in fondaparinux is identical to the AT-binding pentasaccharide sequence present in heparin. Accordingly, fondaparinux also acts indirectly by binding to circulating antithrombin (AT III). It is primarily a selective factor X_a inhibitor, with no activity against thrombin.

Fondaparinux is used in human medicine for the prevention of deep vein thrombosis in patients who have had orthopaedic surgery (hip or knee replacement surgery) as well as for the treatment of deep vein thrombosis and pulmonary embolism. It can also be used as treatment against heparin-induced thrombocytopenia. Similar to other anticoagulants, fondaparinux carries risk of bleeding. Therefore, its use is contraindicated in conditions with increased risk of haemorrhage, such as congenital or acquired bleeding disorders, active ulcerative gastrointestinal disease, haemorrhagic stroke, uncontrolled arterial hypertension, etc. The elimination half-life of fondaparinux in human beings is reported to be about 17 hours. Fondaparinux is commercially supplied as a sterile, preservative-free injectable solution for subcutaneous use.

Idraparinux

Idraparinux is another synthetic oligosaccharide with chemical structure and mechanism of action similar to those of fondaparinux. However, idraparinux is very slowly eliminated with half-life of about 80 hours and is repeated once in a week. It is developed for the treatment and secondary prevention of venous thromboembolism (VTE) as well as the prevention of thromboembolic events associated with atrial fibrillation. Presently it is undergoing clinical trials.

2. Inhibitors of Vitamin K

Inhibitors of vitamin K, often called oral anticoagulants or slow-acting anticoagulants, are a group of drugs which antagonise effects of vitamin K. These drugs differ from heparin and related drugs primarily in their duration of activity and magnitude of effect. These drugs are used occasionally to treat thromboembolic diseases. Studies on their importance in veterinary medicine have focussed primarily on their toxic effects rather than on their therapeutic indications. Inhibitors of vitamin K are active orally and only *in vivo*.

a. Coumarin Derivatives/Coumarins

The coumarins (4-Hydroxycoumarins, coumadins) are synthetic oral anticoagulants derived from the molecule 4-hydroxycoumarin. The series was developed primarily from dicoumarol (bis-hydroxycoumarin) that was identified as the causal agent of a haemorrhagic disease of cattle consuming spoilt sweet clover hay in the USA.

Warfarin

Warfarin sodium is a coumarin derivative anticoagulant, which was originally developed as a haemorrhage inducing rodenticide. Presently, warfarin is also employed clinically as an oral anticoagulant and is considered the prototype of the coumarin-

derived anticoagulants. The commercial preparations of warfarin are a racemic mixture of dextrorotatory (R) and laevorotatory (L) enantiomers. The S-warfarin has five times the potency of the R-isomer with respect to vitamin K antagonism.

Mechanism of action

Warfarin and its congeners are chemically related to vitamin K. Therefore, they apparently behave as competitive antagonists of vitamin K and interfere with the hepatic synthesis of vitamin K-dependent clotting factors viz. Factors II, VII, IX and X, and anticoagulant proteins C and S. Normally these factors undergo vitamin K dependent post-translational modifications, whereby a number of their glutamic acid residues are carboxylated to form γ-carboxyglutamic acid residues. This carboxylation is essential for the ability of clotting factors to bind Ca^{++} and get bound to phospholipid surfaces necessary for coagulation sequence to proceed. In this carboxylation reaction, the vitamin K-dependent carboxylase fixes CO_2 to form the new COOH group on glutamic acid, and vitamin K (reduced form) is converted to vitamin K epoxide (oxidised form). Vitamin K is regenerated from the epoxide by vitamin K epoxide reductase (Figure 35.6). Warfarin and other oral anticoagulant drugs block vitamin K epoxide reductase enzyme and interfere with regeneration of the active form of vitamin K, thereby inhibiting synthesis of prothrombin (Factor II) and Factors VII, IX and X. Therapeutic effects occur when synthesis of clotting factors is reduced by 40-50%.

Warfarin and other oral anticoagulants act as anticoagulants only *in vivo* and not *in vitro* because they act indirectly by interfering with the synthesis of clotting factors. Further, their anticoagulant effect develops gradually over several hours because of the persistence of factors synthesised before drug administration. Levels of clotting factors decline progressively depending on their half-life. Factor VII has the shortest half-life (plasma t½ = 6 hours) and, therefore, is the first factor to become deficient. It is followed by Factor IX (t½ = 24 hours), Factor X (t½ = 40 hours) and prothrombin (t½ = 60 hours).

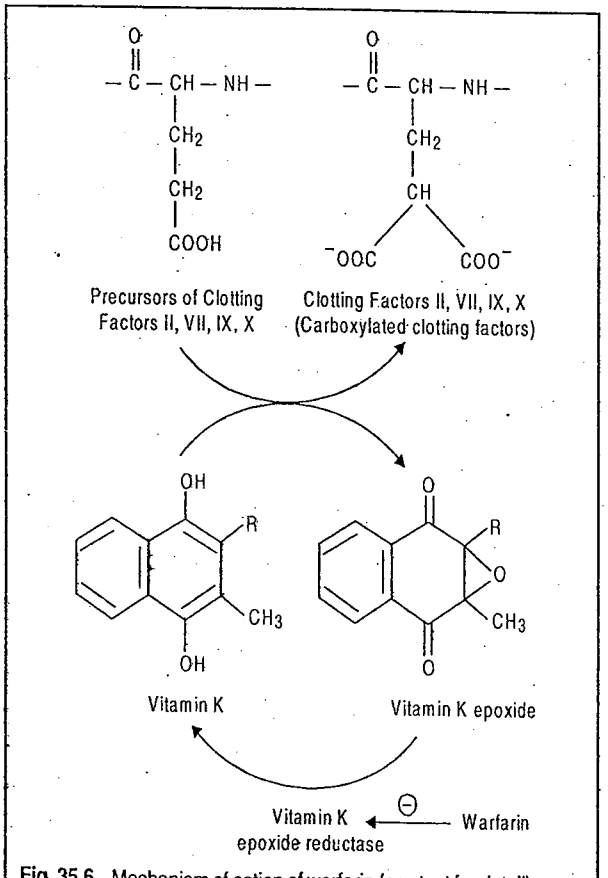


Fig. 35.6. Mechanism of action of warfarin (see text for detail).

Pharmacological effects

Oral administration of warfarin produces gradually developing anticoagulant effect over 1 to 3 days. It has also longer duration of action (4-7 days) because of inhibition of synthesis of vitamin K-dependent clotting factors. Because of the long half-life of some of the coagulation factors (e.g. Factor II), the full antithrombotic effect following inhibition of warfarin therapy is not achieved for several days, even though the prothrombin time may be prolonged soon after administration due to more rapid reduction of factors with a shorter half-life, in particular Factor VII. As serum concentration of anticoagulant protein C decreases more rapidly with warfarin than the clotting factors, there are chances that patient may become hypercoagulable initially (Sometimes heparin therapy is begun for first 2 to 5 days after warfarin therapy is begun to avoid this hypercoagulable state).

As bone also contains some vitamin K-dependent proteins (e.g. osteocalcin, matrix Gla-protein) believed to be involved in mineralization process, prolonged use of warfarin may depress bone formation.

Pharmacokinetics

Warfarin and other vitamin K-dependent anticoagulants are rapidly and completely absorbed from intestine after oral administration. The peak plasma levels occur in 1 hour, but onset of effect takes few hours (6 to 12 hours) with full benefit realised after 2 to 3 days. Warfarin and other coumarin derivatives are highly bound (~99%) to serum albumin, limiting the distribution volume to plasma volume. However, a wide species variation with regard to protein binding has been reported; horses have a greater free (unbound) fraction of the drug than the sheep or swine.

Warfarin is mainly metabolised in liver to inactive metabolites by the cytochrome P-450 system and subsequently conjugated to glucuronide. Excretion mainly occurs in urine and in bile. Warfarin also undergoes an enterohepatic cycle. The plasma half-life of warfarin may be several hours to few days depending on the patient, species and product. For products used as rodenticide, warfarin may have half-life up to 7 days.

Side effects/Adverse effects

The main adverse effect of warfarin use is dose-related haemorrhage, which is due to extension of the desired pharmacological action. Clinical signs are mainly manifested by anaemia, thrombocytopenia, weakness, haematomas and ecchymoses, epistaxis, haematemesis, haematuria, etc. Periarticular or intraarticular haemorrhages can cause lameness, joint swelling and pain. Haemorrhages in brain, spinal cord or subdural spaces are reflected as ataxia, paresis, depression, convulsions, etc. Acute overdosage of warfarin may result in life-threatening haemorrhage.

Treatment: Acute toxicity of warfarin should be treated with blood products and vitamin K_1 (phytomenadione). Vitamin K_1 must be given as long as the anticoagulant is present in the body at toxic levels. Treatment with vitamin K will preclude the therapeutic benefits of warfarin for up to 3 weeks.

Table 35.3: Factors affecting pharmacological action of warfarin.

Type of Action	Examples of Causative Agent(s)
Factors potentiating warfarin action	
1. Displacement of warfarin from plasma proteins	Phenylbutazone, aspirin, etacrynic acid, sulfinpyrazone and long-acting sulphonamides.
2. Inhibition of warfarin metabolism	Phenylbutazone, cimetidine, chloramphenicol, metronidazole, erythromycin, ciprofloxacin, salicylates, imipramine and amiodarone.
3. Inhibition of platelet function	Aspirin and other NSAIDs, and carbenicillin.
4. Inhibition of intestinal vitamin K synthesis	All broad-spectrum antibiotics (e.g. tetracyclines and cephalosporins).
5. Enhanced catabolism of clotting factors	d-Thyroxine, hyperthyroidism and fever.
6. Decreased synthesis of clotting factors	Hepatic diseases, quinine, quinidine, d-thyroxine and paracetamol.
7. Enhancement of warfarin action	Pheniormin, anabolic steroids, quinidine and clofibrate.
Factors suppressing warfarin action	
1. Induction of warfarin metabolism	Microsomal enzyme inducers (e.g. barbiturates and rifampicin).
2. Inhibition of intestinal absorption of warfarin	Colestyramine and sucralfate.
3. Increased synthesis of clotting factors	Vitamin K, oestrogens and pregnancy.
4. Reduced catabolism of clotting factors	Hypothyroidism.

Contraindications and precautions

Warfarin sodium is contraindicated in patients with pre-existent haemorrhage tendency or disease, or in surgery of large open surfaces. It should not be used in purpura, malnutrition, peptic ulcers, acute nephritis, blood dyscrasias, uncontrolled or malignant hypertension, hepatic insufficiency and visceral carcinomas. Warfarin is embryotoxic, therefore it should not be used during pregnancy. During warfarin therapy, it is necessary to carefully monitor coagulation times (particularly prothrombin time), complete blood counts and clinical evidence of bleeding (e.g. occult blood in faeces and urine).

Drug interactions

A variety of factors can influence the anticoagulant activity of warfarin. Because warfarin is highly protein bound, it will be displaced by (or will displace) other drugs which are highly protein bound, causing an increase in free warfarin and possible haemorrhage. On the other hand, some drugs may decrease the anticoagulant effect of warfarin either by increasing its metabolism (e.g. barbiturates) or increasing plasma levels of clotting factors (e.g. vitamin K and oestrogen). Some important factors affecting pharmacological action of warfarin are listed in Table 35.3.

Clinical uses

In veterinary medicine, warfarin is used primarily for the oral, long-term treatment and prevention of recurrence of thrombotic conditions like venous thrombosis and aortic or pulmonary thromboembolism. There is evidence that warfarin can relieve the clinical signs of navicular disease in some horses. Warfarin is more commonly used as an oral rodenticide for controlling rats and mice in residential, industrial and agricultural areas.

Dose

Dogs & cats : 0.1 – 0.2 mg/kg, PO, once daily.

Horses : 0.02 mg/kg, PO, once daily, increasing gradually to desired effect.

Dicoumarol

Dicoumarol (Dicumarol, bis-hydroxycoumarin) is a coumarin anticoagulant that is naturally present in spoiled sweet clover hay. Like warfarin, dicoumarol causes deficiency of prothrombin and other vitamin K-dependent coagulation factors. The pharmacology of dicoumarol is similar to that of warfarin, but it is slowly and unpredictably absorbed orally. Dicoumarol is also reported to show a dose-dependent metabolism i.e. its half-life is

Table 35.4: Comparative features of heparin and warfarin.

Sr. No.	Feature	Heparin	Warfarin
1.	Chemistry	Sulphated glucosaminoglycans (mucopolysaccharides).	Coumarin derivative.
2.	Source	Natural (porcine intestine and bovine lungs).	Synthetic.
3.	Activity	*In vitro* and *in vivo*.	*In vivo* only.
4.	Mechanism of action	Activation of antithrombin III.	Inhibition of vitamin K.
5.	Route of administration	Parenteral.	Oral.
6.	Onset of action	Rapid.	Slow (1 – 3 days).
7.	Duration of action	4 – 6 hours.	4 – 7 days.
8.	Variability in response	Little.	Marked.
9.	Drug interactions	Few and not significant.	Many and significant.
10.	Antagonist	Protamine sulphate.	Vitamin K.
11.	Clinical use	To initiate therapy.	For maintenance.
12.	Laboratory control test	Clotting time, partial prothrombin time.	Prothrombin time.

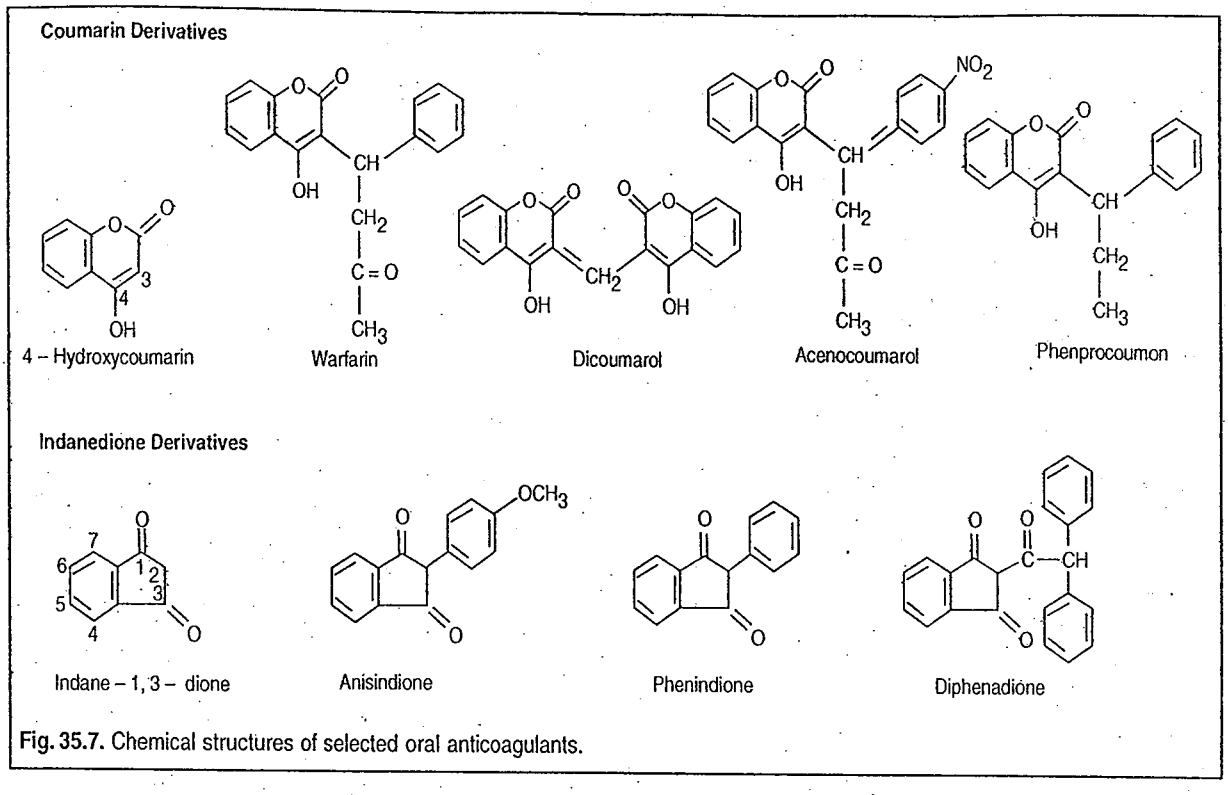


Fig. 35.7. Chemical structures of selected oral anticoagulants.

prolonged at high doses. It is occasionally used as an oral anticoagulant in human medicine.

Other coumarin anticoagulants

Acenocoumarol (Accenocoumarin, nicoumalone), ethylbis-coumacetate, phenprocoumon, coumatetralyl and cyclocoumarol are other 4-hydroxycoumarin derivatives, which are occasionally used as oral anticoagulants in human medicine. Acenocoumarol and ethyl bis-coumacetate act more rapidly but they have shorter duration of action than that of warfarin. Phenprocoumon has a longer plasma half-life (about 5 days) and a longer duration of action (7 to 14 days) than warfarin, but it has somewhat slower onset of action. Coumatetralyl is more commonly used as a rodent poison.

b. Indanedione derivatives

The indanedione (Indandione) oral anticoagulants are derivatives of indane-1, 3-dione. They are structurally related to coumarins and produce anticoagulation by the same mechanism i.e. antagonism of vitamin K. However, these agents have been reported to cause kidney damage, sensitivity reactions and leucopenia in man hence their use has been restricted in therapeutics. Their use in veterinary species has not been reported.

Anisindione

Anisindione is a synthetic indanedione oral anticoagulant that is occasionally used in human medicine. Similar to warfarin, it prevents the formation of active procoagulation factors II, VII, IX and X as well as the anticoagulant proteins C and S in the liver by inhibiting the vitamin K–mediated gamma-carboxylation of precursor proteins. It is similar to warfarin in its pharmacological effects, but may cause a high frequency of untoward effects.

Other indanediones

Phenindione, clorindione and diphenadione are other indanedione anticoagulants which are used rarely in therapy because of more serious non-haemorrhagic toxic effects. Phenindione has been reported to produce serious hypersensitivity reactions in human beings and may also cause agranulocytosis, liver damage and nephropathy. Clorindione is a derivative of phenindione. Diphenadione is mainly used as a rodenticide as a counter to vampire bat predation.

3. Direct Inhibitors of Coagulations Factors

a. Direct Inhibitors of Factor X_a

i. Xabans

Xabans are a novel group of orally administered drugs which directly inhibit the coagulation factor X_a without using antithrombin as a mediator. Several studies have demonstrated usefulness of these compounds as substitutes for the currently administered vitamin K antagonists or low molecular weight heparin. Advantages of xabans lie in the fact that they have a predictable effect, do not require frequent monitoring or re-dosing, are given through the mouth and not by injection and have few (known) drug interactions.

Rivaroxaban

Rivaroxaban is the first available orally active direct factor X_a inhibitor. It is highly selective for coagulation Factor X_a and inhibits both free Factor X_a and Factor X_a bound in the prothrombinase complex. Inhibition of Factor X_a interrupts the intrinsic and extrinsic pathway of the blood coagulation cascade, inhibiting both thrombin formation and development of thrombi. Rivaroxaban does not inhibit thrombin (activated Factor II) and has no effects on platelets.

Rivaroxaban is effective orally and has more predictable anticoagulation and pharmacokinetic profile. After oral administration, it is efficiently absorbed from intestine with peak inhibition of factor X_a occurring in about four hours. In humans, this effect lasts for 8-12 hours, but the activity of Factor X_a may not return to normal even after 24 hours. Rivaroxaban has no major or active circulating metabolites. It undergoes a dual mode of elimination with one-third of the unchanged active drug being

eliminated renally; the remaining two-thirds of the drug are metabolized by the liver. Rivaroxaban is reported to be well tolerated, but chances of bleeding remains. It has been approved for use in human medicine for the prevention of venous thromboembolism (VTE) in patients who have undergone elective total hip replacement or total knee replacement surgery.

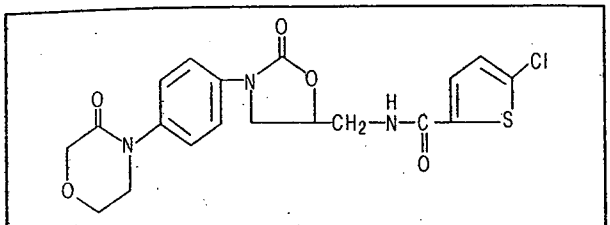


Fig. 35.8. Chemical structure of rivaroxaban

Other drugs

Some other direct inhibitors of factor X_a include apixaban, betrixaban, edoxaban and otamixaban. Apixaban, betrixaban and edoxaban are presently undergoing phase III trials in the prevention of venous thromboembolism. Otamixaban is currently being developed as a treatment for acute coronary syndrome.

b. Direct Inhibitors of Coagulation Factor II

i. Hirudin and Derivatives

Hirudin

Hirudin is a naturally occurring anticoagulant protein present in the salivary glands of medicinal leeches such as *Hirudo medicinalis*. Full length hirudin is made up of 65 amino acids. Hirudin is considered as the most potent natural inhibitor of thrombin. Unlike antithrombin III, hirudin binds to and inhibits only the activity of thrombin with a specific activity on fibrinogen. Therefore, it prevents not only clot formation but also dissolves the clots and thrombi (i.e. it has a thrombolytic activity). In some aspects, hirudin has advantages over more commonly used anticoagulants and thrombolytics, such as heparin, as it does not interfere with the biological activity of other serum proteins and can also act on complexed thrombin. Hirudin is used therapeutically in the treatment of various blood coagulation disorders, skin hematomas and superficial varicose veins. It is used as either an injection or a topical application cream.

As it is difficult to extract large amounts of hirudin from natural sources, so methods for producing and purifying this anticoagulant protein using recombinant biotechnology has been developed. Furthermore, natural hirudin contains a mixture of various isoforms of the protein, but the recombinant techniques can produce homogeneous preparations of hirudin. These limitations with hirudin has led to the development and marketing of a number of hirudin based anticoagulant pharmaceutical products.

Bivalirudin

Bivalirudin is the synthetic congener of the naturally occurring drug hirudin. It is highly specific and reversible direct inhibitor of thrombin (Factor II). It inhibits both circulating and clot-bound thrombin, while also inhibiting thrombin-mediated platelet activation and aggregation. However, it does not bind to other plasma proteins or red blood cells and has no action on the platelet aggregation. Unlike heparin, it has a predictable antithrombotic response.

Bivalirudin has a quick onset of action and a short half-life of about 30 minutes. It is cleared from plasma by a combination of renal mechanisms and proteolytic cleavage. These properties make bivalirudin an ideal alternative to heparin. Bivaluridin possesses risk of bleeding, especially in susceptible individuals, but carries no risk of thrombocytopenia. Bivalirudin is contraindicated in patients with active major bleeding and hypersensitivity to bivalirudin or its components. It is intended for IV use only and is supplied as a sterile, lyophilized product in single-use, glass vials.

Desirudin

Desirudin is a synthetic recombinant hirudin produced in yeast cells, differing from the naturally occurring molecule by the absence of a sulphate group on one amino acid. It is highly selective and almost irreversible inhibitor of human thrombin, with no action on other components of the haemostatic system. After subcutaneous administration, desirudin is almost completely absorbed with onset of action in 30 minutes. It has elimination half-life of 2 to 3 hours and duration of action about 6 hours. Desirudin is chiefly eliminated unchanged by the kidneys. Bleeding complications are the most commonly seen adverse effects. Desirudin's anticoagulant effect is measured by prolongation of thrombin time (TT) and activated partial thromboplastin time (aPTT). It has been approved for use in human medicine for the prevention of deep vein thrombosis in patients undergoing elective hip and knee replacement surgery.

Lepirudin

Lepirudin is a recombinant hirudin congener, derived from yeast cells. Chemically it differs from hirudin extracted from *Hirudo medicinalis* by the substitution of leucine for isoleucine at the N-terminal end of the molecule and the absence of a sulphate group on the tryosine at position 63. Its mechanism of action and pharmacological properties are similar to those of hirudin. Lepirudin may be used as an anticoagulant when heparins (unfractionated or low molecular weight) are contraindicated because of heparin-induced thrombocytopenia.

ii. Miscellaneous Direct Thrombin Inhibitors

Argatroban

Argatroban is a novel anticoagulant from the class of the direct thrombin inhibitors. It acts solely by inhibiting the actions of thrombin, with no/negligible action on other coagulation factors. It has been approved for human use for prophylaxis or treatment of thrombosis in patients with heparin-induced thrombocytopenia. Argatroban is metabolized in the liver and has a half life of about 50 minutes. Because of its hepatic metabolism, it may be used in patients with renal dysfunction. Its activity is monitored by partial thromboplastin time (PTT).

Other drugs

Dabigatran and ximelagatran are two other direct acting thrombin inhibitors. Dabigatran has been recently approved for the treatment and prevention of thromboembolic disease. Ximelagatran is currently undergoing clinical trials.

4. Miscellaneous Anticoagulants

Defibrotide

Defibrotide is the sodium salt of a mixture of single-stranded oligodeoxyribonucleotides derived from porcine mucosal DNA. It has been shown to have antithrombotic, anti-inflammatory and anti-ischemic properties. It produces anticoagulant and

antithrombotic actions through multiple mechanisms. It prevents formation of blood clots and also helps dissolve blood clots by increasing levels of prostaglandin I_2 and E_2. Prostaglandin I_2 relaxes the smooth muscle of blood vessels and prevents platelets from adhering to each other, while prostaglandin E_2 at certain concentrations also inhibits platelet aggregation. Defibrotide also appears to increase tissue plasminogen activator (tPA) function and decrease activity of tissue plasminogen activator inhibitor. Unlike heparin and warfarin, defibrotide appears to have a relatively mild anticoagulant activity, which may be beneficial in the treatment of patients at high risk of bleeding complications.

Defibrotide has been approved for use in human medicine for various coagulative disorders such as hepatic veno-occlusive disease, peripheral obliterative arterial disease and thrombophlebitis. In very high doses defibrotide is useful as treatment of acute myocardial infarction. The drug may also be used for the pre- and post-operative prophylaxis of deep venous thrombosis and can replace the heparin use during haemodialytic treatments. Defibrotide has been used with antithrombin III.

Drotrecogin alpha

Drotrecogin alpha (Anticoagulant protein C) is a recombinant form of human activated protein C (rhAPC) that has anti-thrombotic, anti-inflammatory and profibrinolytic properties. The specific mechanisms by which drotrecogin exert its pharmacological effects is not completely understood, but it is suggested that it exerts an antithrombotic effect by inhibiting factors V_a and $VIII_a$. It also has indirect profibrinolytic activity by inhibiting plasminogen activator inhibitor-1 (PAI-1). Its anti-inflammatory effect is related to inhibition of tumour necrosis factor production, by blocking leukocyte adhesion to selectins and by limiting the thrombin-induced inflammatory responses within the microvascular endothelium. Drotrecogin is indicated primarily for the reduction of mortality in human patients with severe sepsis and acute respiratory distress syndrome. Activated drotrecogin alpha is designated as orphan medicinal product.

Antithrombin III (Human)

Antithrombin III (Human), commercially marketed as Thrombate III, is a sterile, nonpyrogenic, stable, lyophilized preparation of purified human antithrombin III. It is indicated for the treatment of patients with hereditary antithrombin III deficiency in connection with surgical or obstetrical procedures or when they suffer from thromboembolism.

Ramatroban

Ramatroban is a thromboxane A_2 (TXA_2) receptor antagonist. It is known that thromboxane A_2 (TXA_2) contributes to various diseases such as bronchial asthma, ischemic heart disease, cerebrovascular disorders and allergic rhinitis. It is indicated for the treatment of coronary artery disease and in asthma (see also Chapter 29).

II. THROMBOLYTICS/FIBRINOLYTICS

Thrombolytics/Fibrinolytics are drugs used to dissolve blood clots/thrombi after they have formed. These drugs primarily increase the activity of plasmin (fibrinolysin), an endogenous compound that is responsible for dissolving clots. As plasmin is a relatively non-specific protease, therapy with thrombolytic agents tends to dissolve both unwanted thrombus (pathological thrombus) and fibrin of a beneficial haemostatic plug (physiological thrombus) at the site of vascular injury. Therefore, the thrombolytic drugs are toxic, producing haemorrhage as a major side effect. Thrombolytics are used in myocardial infarction, thromboembolic strokes, deep vein thrombosis and pulmonary embolism to clear a blocked artery and avoid permanent damage to the perfused tissue. A less frequent use is to clear blocked catheters which are used in long-term medical therapy.

1. PLASMINOGEN ACTIVATORS

Tissue plasminogen activator

Tissue plasminogen activator (tPA), a serine protease found on endothelial cells of blood vessels, is involved in the breakdown of blood clots. It catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown. Its action is more selective towards the plasminogen bound to fibrin and has low affinity for free plasminogen. Thus it confines fibrinolysis to the formed thrombus, without unwanted degradation of other proteins. Tissue plasminogen activator is now obtained by recombinant DNA technology (recombinant tissue plasminogen activator, rtPA). Important rtPA used as thrombolytics include alteplase, reteplase, tenecteplase and monteplase.

Alteplase

Alteplase is a tissue plasminogen activator produced by the recombinant DNA technology. It is synthesized using the complementary DNA (cDNA) for natural human tissue-type plasminogen activator obtained from a human melanoma cell line. It exists as a sterile, purified glycoprotein of 527 amino acids. Alteplase possesses characteristics similar to the naturally occurring tissue plasminogen activator and catalyzes the conversion of plasminogen into plasmin. Alteplase seems to be superior to streptokinase and urokinase in dissolving older clots.

Alteplase is used in diseases which feature blood clots, such as pulmonary embolism, myocardial infarction and stroke. Because alteplase works on the clotting system, it is recommended only in embolic or thrombotic stroke. Its use is contraindicated in hemorrhagic stroke and head trauma. The most frequent adverse reaction associated with alteplase in all approved indications is bleeding. Allergic-type reactions e.g. anaphylactoid reaction, laryngeal oedema, angioedema, rash and urticaria have also been reported with alteplase. Adverse effects of alteplase in cats include haemorrhage, heart failure and death from reperfusion injury.

Dose

For aortic thromboembolism

Cats	:	0.25 – 1 mg/kg/hour, IV infusion, for a total dose of 1 – 10 mg/kg.
Dogs	:	0.01 μg/kg/min, IV infusion for 30 minutes.

Reteplase

Reteplase is a recombinant non-glycosylated form of human tissue plasminogen activator, which has been modified to contain 357 of the 527 amino acids of the original protein. It is produced in the bacterium *Escherichia coli*. Reteplase is similar in action to the alteplase, but has longer half-life of 13–16 minutes. Reteplase also binds fibrin with lower affinity than alteplase, improving its ability to penetrate into clots. The incidence of bleeding as adverse effects with reteplase is also comparatively lower than that seen with alteplase. It is available for IV injection only and is indicated for use in the management of acute myocardial infarction (AMI) in human beings.

Tenecteplase

Tenecteplase is another recombinant fibrin-specific plasminogen activator that is derived from native tPA by modifications using an established mammalian cell line (Chinese Hamster Ovary cells). It binds to the fibrin component of the thrombus (blood clot) and selectively converts thrombus-bound plasminogen to plasmin, which degrades the fibrin matrix of the thrombus. Tenecteplase has a higher fibrin specificity and greater resistance to inactivation by its endogenous inhibitors compared to natural tPA. Tenecteplase is indicated in thrombolytic treatment of the acute phase of myocardial infarction (AMI).

Streptokinase

Streptokinase (SK) is a protein obtained from Group C β-haemolytic streptococci. It has a molecular weight of 47 kDa. It has no intrinsic enzymatic activity. The SK is used as an effective and inexpensive clot-dissolving drug in some cases of myocardial infarction and pulmonary embolism.

Mechanism of action

Streptokinase primarily acts as a plasminogen activator and enhances the production of plasmin. Streptokinase itself is inactive, but it forms an active non-covalent 1: 1 complex with plasminogen. This produces a conformational change that exposes the active site on complexed plasminogen, which then acts on free plasminogen and converts it to the active enzyme plasmin. Extra production of plasmin caused by streptokinase breaks down unwanted blood clots or fibrin plugs. In addition to the lysis of fibrin plugs, plasmin catalyses degradation of some other plasma proteins, including several clotting factors (e.g. fibrinogen, and Factors V and VII). The streptokinase-plasminogen complex in not inhibited by α_2-antiplasmin.

Pharmacological effects

Intravenous administration of streptokinase produces intense thrombolytic effect in the body. In general, venous thrombi are lysed more easily than arterial thrombi. Streptokinase and other thrombolytic drugs are more effective against the recent thrombi and have little effect on thrombi which are more than 3 days old. Streptokinase, like other fibrinolytic agents, can cause bleeding by dissolving physiological thrombus and by degrading some clotting factors.

Pharmacokinetics

Pharmacokinetic studies on streptokinase in domestic animals are limited. The drug is used either by local or systemic route. Intravenous infusion gives rapid onset of action. As anti-streptococcal antibodies present due to past infections inactivate considerable fraction of the initial dose of streptokinase, a loading dose of drug is usually administered in the beginning. The half-life of streptokinase in man is about 30- 80 minutes.

Side effects/Adverse effects

Streptokinase is a foreign protein and is antigenic. Therefore, its systemic use can cause hypersensitivity and anaphylaxis, especially when used second time in a patient. Fever, rashes, hypotension and arrhythmias are reported in human beings. Streptokinase may precipitate bleeding by dissolving haemostatic plug. With high doses of streptokinase, plasminogen depletion and streptokinase resistance may occur. These can be overcome by administering fresh plasma. Overdose of streptokinase can be treated with aminocaproic acid.

Clinical uses

Streptokinase is used primarily in human medicine in acute pulmonary embolism, venous and arterial thrombosis, and acute myocardial infarction. For myocardial infarction, it is infused IV over 60 minutes, whereas for severe venous thrombosis or pulmonary embolism longer infusions (up to 72 hours) may be required. In veterinary medicine, streptokinase is used mainly locally as a powder, infusion or irrigation in the treatment of wounds which do not respond to antibacterial therapy. These include burns, ulcers, chronic eczemas, ear haematomas, otitis externa, osteomyelitis, chronic sinusitis, or other chronic lesions. It is also used parenterally in the treatment of ulcers, eczema, oedema, dermatitis, haematoma, trauma and pneumonia. It can be useful clinically for reduction of occurrence of post-operative adhesions. It may be considered useful for the treatment of feline thromboembolic disease, but clinical efficacy is not clear.

Dose

Dogs & cats : 5,000 – 10,000 Units (total), IM or IV in 1 or 2 divided doses for up to 5 days.

Large animals : 5,000 – 10,000 Units/45 kg body wt., IM or IV in 1 or 2 divided doses daily for up to 5 days.

Urokinase

Urokinase, also called urokinase-type plasminogen activator (uPA), is a serine protease enzyme that activates plasminogen directly. It was originally isolated from human urine, but is now obtained from cultured human kidney cells. Like streptokinase, it lacks fibrin specificity and therefore readily induces a systemic lytic state. Urokinase is very expensive, so it is used mainly in human medicine in patients who are sensitive to streptokinase. Bleeding complications are the most important side effects of this drug therapy. In veterinary medicine, it can be used in the treatment of selected chronic wounds which do not respond to other therapy. It may also be used to prevent serosal post-operative adhesions in dogs when administered as an intraperitoneal lavage.

Dose

To prevent post-operative lesions

Dogs : 5,000 – 10,000 Units/kg, intraperitoneal lavage.

Streptodornase

Similar to streptokinase, streptodornase is obtained from streptococcal organisms and it activates plasminogen. Its pharmacological effects and clinical uses are similar to those of streptokinase. Streptodornase is also available in combination with streptokinase in concentration of 100,000 streptokinase units, 25,000 streptodornase units and 500 plasminogen units. The antibacterial agents are generally added to the enzyme preparations.

Anistreplase

Anistreplase (Anisoylated plasminogen-streptokinase activator complex, APSAC) is a complex of human plasminogen and streptokinase, which has been rendered inactive by the introduction of p-anisoyl group. The acyl group is hydrolysed *in vivo*, allowing the complex to bind to fibrin prior to activation; thus the complex is semi-selective for lysis at the clot site. Indications and side effects of anistreplase are the same as described earlier for other plasminogen activators. High cost and adverse effects preclude its use in animals.

Saruplase

Saruplase is the non-glycosylated form of single chain urokinase-type plasminogen activator (u-PA) produced by recombinant technique in *Escherichia coli*. It possesses plasminogen activating properties with characteristics similar to those of urokinase.

2. Other Drugs

Ancrod

Ancrod is a proteolytic enzyme that is derived from the venom of the Malayan pit viper. Ancrod may also be found in the venom of many venomous snakes (crotalids, elapids and viperids) in general, but the Malayan pit viper is most suitable due to a high concentration of ancrod in its venom. It possesses fibrinolytic action that produces an anticoagulant effect. The exact structure and chemical data such as molecular weight of ancrod are unknown, but it has been elaborated that its fibrinolytic actions are flavin adenine dinucleotide (FAD) dependent and that the substance possesses apoptotic properties. It also indirectly inhibits aggregation, adhesion and release of thrombocytes mediated through the action of a fibrinogen degradation product (FDP). The half-life of ancrod is 3 to 5 hours and the drug is cleared from blood plasma mainly via urine.

Hypersensitivity reactions such as local or generalized skin reactions (rash and urticaria), bleeding and mild pain at injection site may occur with ancrod injection. Drug resistance due to appearance of neutralizing antibodies to ancrod with partial or total loss of ancrod activity has been observed. It should not be used during pregnancy as the defibrinogenation mechanism of ancrod might be expected to interfere with the normal implantation of the fertilized egg. Ancrod is occasionally used in human medicine for deep vein thrombosis in patients who develop thrombocytopenia or hypersensitivity reaction to heparin. Bleeding and occasional hypersensitivity reactions are main adverse effects. Ancrod is recommended for subcutaneous injection and intravenous infusion.

Fibrinolysin

Fibrinolysin is an enzyme derived from bovine plasma or extracted from cultures of certain bacteria. It is used locally only and exclusively together with the enzyme desoxyribonuclease (extracted from bovine pancreas). Fibrinolysin and desoxyribonuclease both act as lytic enzymes and their action is synergistic. Fibrinolysin attacks and inactivates fibrin molecules occurring in undesirable exudates e.g. in superficial wounds and burns, while desoxyribonuclease targets and destroys DNA. Fibrinolysin is mainly used for wound cleaning to assist healing of minor burns, superficial surgical wounds, ulcers and hematoma.

Brinase

Brinase (Brinolase) is a plasmin like enzyme derived from *Aspergillus oryzae*. It has fibrinolytic and proteolytic activity and has been used for certain arterial obliterative disease conditions.

III. ANTITHROMBOTICS

Antithrombotics (Antiplatelet drugs) are agents which inhibit platelet activation and/or aggregation. As platelets provide the initial haemostatic plug by first sticking to the damaged vessel wall and then to each other (aggregation), antiplatelet drugs are useful in the prophylaxis of thromboembolic disorders. Unlike anticoagulant drugs which are more successful in venous thrombosis, antithrombotic drugs are more useful in the prevention of arterial thrombosis because platelet mass is the main constituent of arterial thrombus (white thrombus). Antiplatelet drugs do not dissolve existing thrombi, but they can help to inhibit their growth and prevent recurrence. Feline cardiomyopathy and canine heartworm infection are examples of disease processes which are often complicated by arterial thrombus.

1. Cyclooxygenase Inhibitors

Aspirin

Aspirin is a non-steroidal anti-inflammatory drug (NSAID) that irreversibly inactivates cyclooxygenase (COX) enzyme to produce its pharmacological effects. This reduces synthesis of all prostanoids including that of thromboxane A_2, a potent vasoconstrictor and inducer of platelet aggregation. Low doses of aspirin are more effective in reducing platelet aggregation than high doses because in low doses it inhibits synthesis of TXA_2 only, while in high doses it also blocks synthesis of prostacyclin, a platelet anti-aggregator, which reduces the overall antiaggregatory effect of the drug (see for detail Chapter 30). In addition to its inhibitory effects on COX, aspirin is reported to irreversibly acetylate thromboxane synthase, the specific enzyme responsible for synthesis of thromboxanes. Inhibition of thromboxane produces antiaggregatory effect on platelets with a useful antithrombotic effect.

Aspirin has been used clinically to reduce further platelet aggregation after a thromboembolic event. In veterinary medicine, aspirin remains a component of therapy directed toward prevention and treatment of arterial thrombosis, including severe pulmonary arterial thrombosis associated with heart worm disease. The pharmacology of aspirin has been given in Chapter 30.

Dose

For antiplatelet effect

Dogs (normal dog)	:	0.5 mg/kg, PO, 2 times daily.
(Heart worm infested dog)	:	5 – 10 mg/kg, PO, every 24 to 48 hours.
Cats	:	75 mg (total dose), PO, every 48 to 72 hours.

Other drugs

Other cyclooxygenase inhibitors occasionally used as antithrombotic drugs include aloxiprin, carbasalate calcium, indobufen and triflusal. Aloxiprin (aluminium acetylsalicylate) and carbasalate calcium (mixture of calcium acetylsalicylate and urea) have platelet anti-aggregation activities similar to those of aspirin. Indobufen acts as a reversible cyclooxygenase inhibitor and a platelet aggregation inhibitor. Triflusal prevents platelet aggregation by dual mechanisms i.e. by inhibiting cyclooxygenase enzyme and blocking phosphodiesterase enzyme.

2. Adenosine Diphosphate (ADP) Receptor Inhibitors

a. Thienopyridines

Thienopyridines are a newer class of antithrombotic drugs which mediate their anti-platelet activity through inhibition of adenosine diphosphate (ADP) P2Y receptors. Important drugs belonging to this class include clopidogrel, prasugrel and ticlopidine.

Clopidogrel

Clopidogrel is a novel thienopyridine antiplatelet agent. It acts by specifically and irreversibly inhibiting the $P2Y_{12}$ subtype of ADP receptor, which is important in aggregation of platelets

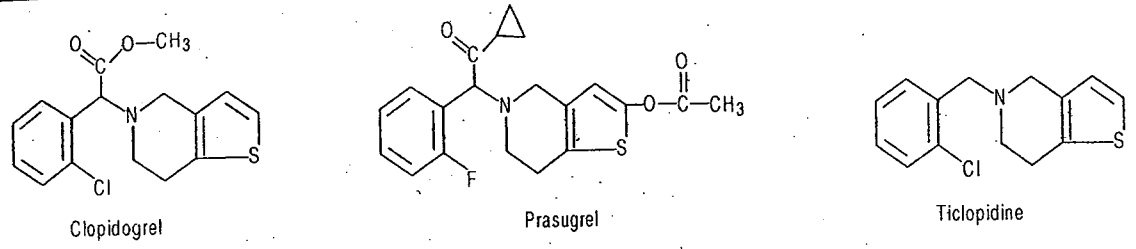


Fig. 35.9. Chemical structures of important adenosine diphosphate receptor inhibitors.

and cross-linking by the protein fibrin. The blockade of this receptor inhibits platelet aggregation mainly by preventing activation of the glycoprotein IIb/IIIa pathway. Clopidogrel is basically a prodrug that is rapidly absorbed after oral administration and binds reversibly to plasma proteins up to 98%. In liver it is rapidly converted into an active metabolite by cytochrome P450 enzymes, including CYP2C19 with peak plasma levels of the main circulating metabolite occurring approximately one hour after dosing. In human patients, platelet inhibition can be demonstrated two hours after a single dose of oral clopidogrel, but the onset of action is relatively slow.

Important adverse effects of clopidogrel include haemorrhage, severe neutropenia and thrombotic thrombocytopenic purpura (TTP). Clopidogrel shows interactions with several drugs including proton pump inhibitors, phenytoin and tissue plasminogen activators (e.g. alteplase and streptokinase). It should not be used along with NSAIDs due to increased chances of GI bleeding. Clopidogrel has been recommended for use in human medicine to inhibit blood clots in coronary artery disease, peripheral vascular disease and cerebrovascular disease.

Prasugrel

Prasugrel is another thienopyridine antiplatelet with actions similar to those of clopidogrel. Compared to clopidogrel, prasugrel inhibits adenosine diphosphate–induced platelet aggregation more rapidly, more consistently and to a greater extent. As a result, platelet aggregation and clot retraction are inhibited. Similar to clopidogrel, prasugrel is a prodrug and is rapidly metabolized to a pharmacologically active metabolite and inactive metabolites. The active metabolite has an elimination half-life of about 7 hours. It is used for the reduction of thrombotic cardiovascular events. Hypotension, nausea, diarrhoea and GI bleeding are some important side effects.

Ticlopidine

Ticlopidine is a thienopyridine antiplatelet drug that has been studied extensively in human patients with thromboembolic diseases. It mediates its action through adenosine diphosphate (ADP) receptor and alters the fibrinogen activity in such a way that fibrinogen in not able to bind to the activated platelets. It has no effect on platelet cAMP or TXA_2, but prolongs bleeding time. After oral ingestion, it is extensively bound to plasma proteins. Its action is low in onset, taking 3 to 7 days to reach maximal effect. It is mainly used in human patients who are intolerant to aspirin. Unwanted effects like prolonged bleeding, nausea and diarrhoea, and neutropenia has restricted its regular use.

b. Other drugs

Ticagrelor

Ticagrelor is a novel non-thienopyridine antiplatelet drug with mechanism of action similar to clopidogrel, prasugrel and ticlopidine. However, it blocks the adenosine diphosphate $P2Y_{12}$ receptors reversibly. Moreover, it is a direct acting drug and does not need hepatic activation, which could reduce the risk of drug interactions. Ticagrelor is currently approved for use in human patients in European countries.

3. Glycoprotein IIb/IIIa Inhibitors

Glycoprotein IIb/IIIa inhibitors are a new class of potent platelet aggregation antagonists which act by blocking glycoprotein IIb/IIIa receptors present on the surface of platelets. As discussed earlier, the glycoprotein IIb/IIIa complex functions as a receptor for fibrinogen and von Willebrand factor through which agonists like collagen, thrombin, thromboxanes and adenosine diphosphate, etc. induce platelet aggregation. Important drugs of the class include abciximab, eptifibatide and tirofiban.

Abciximab

Abciximab (previously known as c7E3 Fab) is the Fab fragment of a chimeric monoclonal antibody against glycoprotein IIb/IIIa. Abciximab has a short plasma half life of 10-30 minutes, but due to its strong affinity for the glycoprotein IIb/IIIa receptor on the platelets, the platelet aggregation remains inhibited for several days. In practice, platelet aggregation gradually returns to normal about 96 to 120 hours after discontinuation of the drug. Abciximab is used mainly during and after coronary artery procedures like angioplasty to prevent platelets from sticking together and causing thrombus (blood clot) formation within the coronary artery. Important side effects of abciximab are related to its anti-platelet effects and include increased risk of haemorrhage.

Other drugs

Eptifibatide and tirofiban are other glycoprotein IIb/IIIa inhibitors, developed as alternatives to abciximab. Eptifibatide is a cyclic heptapeptide derived from a protein found in the venom of the south-eastern pygmy rattlesnake (*Sistrurus miliarius barbouri*), whereas tirofiban is a synthetic non-peptide drug.

4. Thromboxane Inhibitors

Dipyridamole

Dipyridamole is an antithrombotic and a vasodilator drug. Its antithrombotic effect is mediated through inhibition of enzyme thromboxane synthase, which decreases synthesis of thromboxane TXA_2 and thus prevents the platelet aggregation (see also Chapter 34). It also elevates endogenous cAMP in the platelets by inhibiting phosphodiesterase enzyme. Other actions may include blockade of cellular reuptake of adenosine into platelets, red blood cells and endothelial cells leading to increased extracellular concentrations of adenosine. When used alone, dipyridamole minimally affects platelet functions, but with aspirin it acts synergistically to inhibit platelet activity. It also improves the response to warfarin along with which it is used in human medicine

to decrease the incidence of thromboembolism in patients with prosthetic heart valves.

Dazoxiben

Dazoxiben is an inhibitor of thromboxane synthase that selectively decreases thromboxane A_2 (TXA_2) synthesis. It also increases prostacyclin (PGI_2) synthesis as a consequence of diversion of endoperoxide intermediates from TXA_2 synthesis to PGI_2 synthesis. However, it does not inhibit platelet aggregation significantly when given alone, but only when used in combination with aspirin.

Picotamide

Picotamide, a derivative of methoxy-isophtalic acid, is a platelet aggregation inhibitor. It acts by dual mechanisms i.e. by inhibiting the enzyme thromboxane-A synthase and blocking thromboxane A_2 (TXA_2) receptors. The reduction of circulating platelet-derived TXA_2 levels induced by picotamide inhibits platelet aggregation, which is considered useful in some thromboembolic disorders.

Terutroban

Terutroban is a selective antagonist of the thromboxane receptor. It blocks thromboxane induced platelet aggregation and vasoconstriction. Terutroban is currently undergoing clinical trials.

5. Miscellaneous Drugs

Cilostazol

Cilostazol is a phosphodiesterase inhibitor with significant inhibitory effects of platelet aggregation. Inhibition of phosphodiesterase enhances the intracellular concentration of cAMP. This results in an increase in the active form of Protein kinase A (PKA), which is directly related with an inhibition in platelet aggregation and an arterial vasodilator effects. Cilostazol has been recommended for the treatment of some muscular pains due to cramps, numbness or fatigue.

Sulfinpyrazone

Sulfinpyrazone (Sulphinpyrazone) is a uricosuric drug related to phenylbutazone. It inhibits cyclooxygenase enzyme and thereby blocks production of all prostanoids including TXA_2 synthesis in the platelets. Although selectivity for platelets has not been demonstrated, sulfinpyrazone blocks the release reactions in platelets, increases their survival time in thromboembolic diseases, and reduces platelet adhesion to collagen. Clinically, sulfinpyrazone has not gained popularity as antiplatelet drug.

Other drugs

Cloricromen and ditazole are two other less commonly used platelet aggregation inhibitors. Simultaneous use of timolol, a beta-adrenoceptor blocker, synergises the action of low-dose aspirin. Some prostaglandin I_2 (Prostacyclin) analogues such as beraprost, iloprost and treprostinil possess significant platelet aggregatory and antithrombotic effects, but are less used clinically for these effects.

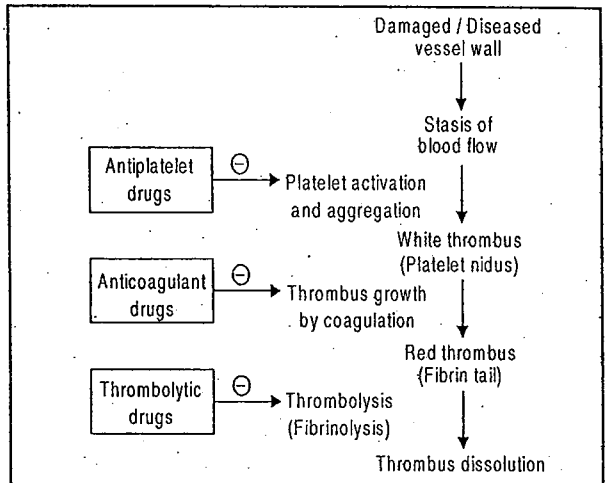


Fig. 35.10. Sequence of events leading to thrombus formation and dissolution.

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CHAPTER 36 HAEMATOPOIETIC DRUGS

Haematinics/Anti-anaemic drugs
- Anaemia
- Classification
- Nutritional agents
  - Minerals
  - Vitamins
- Haematopoietic growth factors
  - Erythroid growth factors
  - Myeloid growth factors
    - Granulocyte-colony stimulating factor (G-CSF)
    - Granulocyte/macrophage colony stimulating factor (GM-CSF)
    - Stem cell factor (SCF)
    - Interleukins
- Miscellaneous drugs

Haematopoietic (Haemopoietic) agents are drugs which assist in or stimulate the production of blood cells. Haematopoiesis is a complex phenomenon that occurs through differentiation of stem cell, which is formed early in embryonic life (see Figure 36.3). The main components of haematopoietic system are blood and bone marrow, with spleen and liver as important accessory organs. In addition, kidney and some other tissues synthesize and release certain growth factors, which regulate the production of blood cells. Haematopoiesis essentially requires adequate supply of some minerals and vitamins needed for synthesis of blood cell and certain haematopoietic growth factors needed for stimulating production of blood cells.

HAEMATINICS / ANTI-ANAEMIC DRUGS

Haematinics are agents which are required for or help in the formation of blood. They improve quality of blood mainly by increasing the number of erythrocytes and/or the concentration of haemoglobin concentration. Haematinics are used clinically for the treatment and prevention of anaemia and are often called antianaemic drugs.

Anaemia

Anaemia is defined as deficiency of erythrocytes or haemoglobin per unit volume of blood. Anaemia occurs when the balance between production and destruction of red blood cells is disturbed. This may occur either due to excessive loss of blood (e.g. haemolysis and haemorrhage) called primary anaemia, or due to impaired cell formation called secondary anaemia (Table 36.1). There are several other ways to classify anaemia including on the basis of morphology of RBCs, underlying etiological mechanisms and discernible clinical spectra. The symptoms of anaemia, irrespective of its cause and type, are due to diminished oxygen carrying capacity of the blood leading to impaired tissue oxygenation. Clinically, anaemia is characterised by pallor, muscular weakness, dyspnoea, increased rate and force of heartbeats, easy fatigability and poor exercise tolerance. Removal of primary cause is essential for the treatment of primary anaemia. If degree of anaemia is severe, transfusion of whole blood may be done. Antianaemic drugs are used mainly to supplement deficiencies of nutritional factors necessary for normal production of red blood cells. Haematopoietic growth factors, particularly erythropoietin, are being also used against some specific types of anaemia.

Table 36.1: Types of anaemia and their causative agents.

Type of Anaemia	Examples of Causative Agents/Diseases
I. Primary Anaemia	
1. Haemorrhagic	
a. Chronic parasitic infections	Coccidiosis and hook worms.
b. Gastrointestinal lesions	Ulcerative colitis and abomasal ulcers.
c. Defective coagulation	Haemophilia A and deficiency of vitamin K.
d. Defective endothelium	Purpurea haemorrhagica.
2. Haemolytic	
a. Infectious	Babesiosis, theileriosis and bacillary haemoglobinuria.
b. Non-infectious	Phenothiazine toxicity, incompatible blood transfusion and autoimmune reactions.
II. Secondary Anaemia	
1. Pathological diseases	Chronic interstitial nephritis, hypothyroidism and tuberculosis.
2. Deficiency of nutrients	Iron, copper, cobalt, folic acid and vitamins.
3. Toxicity	Sulphonamides, chloramphenicol and arsenic.

Classification

Anti-anaemic drugs can be arbitrarily classified into following groups:

I. Nutritional agents
  1. Minerals
     e.g. iron, cobalt and copper.
  2. Vitamins
     e.g. vitamin B_{12}, folic acid, pyridoxine, riboflavin and ascorbic acid.

II. Haematopoietic growth factors
  1. Erythroid growth factors
     e.g. erythropoietin (epoetin alpha and epoetin beta) and darbepoetin alfa.
  2. Myeloid growth factors
     a. Granulocyte-colony stimulating factor (G-CSF)
        e.g. filgrastim, pegfilgrastim and lenograstim.
     b. Granulocyte/Macrophage colony stimulating factor (GM-CSF)
        e.g. sargramostim and molgramostim.
     c. Stem cell factor (SCF)
        e.g. ancestim.
     d. Interleukins
        e.g. oprelvekin.

III. Miscellaneous agents
  1. Anabolic steroids
     e.g. nandrolone.

I. NUTRITIONAL AGENTS

1. Minerals

Iron

Iron is a metallic element with the symbol Fe (Latin: *ferrum*) and atomic number 26. Iron is essentially required for the synthesis of haemoglobin. Deficiency of iron often leads to anaemia of different degrees. Iron deficiency anaemia is one of the most common types of anaemias.

Distribution

Iron occurs in body in haemoglobin, myoglobin, transferrin, ferritin and iron containing porphyrins and is an essential component of enzymes such as catalase, peroxidase and various cytochromes. About 65-70% of total body iron is present in haemoglobin, 4-9% in myoglobin and 2% in cytochrome and electron transport system. The remaining 25% to 30% is stored as either ferritin, the soluble form of iron stores, or haemosiderin, the insoluble form (Table 36.2).

Haemoglobin: Haemoglobin is made up of four protein chains subunits (globins), each of which contains one haem moiety. Haem consists of a tetrapyrrole porphyrin ring containing ferrous (Fe^{++}) iron (Figure 36.1). Each haem group can carry one oxygen molecule, which is bound reversibly to the Fe^{++} and a histidine residue in the particular globulin chain to which the haem is linked. The reversible binding is the basis of oxygen transport in the body.

Table 36.2: Iron distribution in the body.

Protein	Tissue	Approximate Iron content (%)	Available for haematopoiesis
I. Essential Iron (70-75%)			
1. Haemoglobin	Erythrocytes	65 – 75	Recycled
2. Myoglobin	Muscles	4 – 9	No
3. Enzymes	Liver and other tissues	0.3	No
4. Transferrin	Plasma and extracellular fluid	0.2	Yes, in transport
II. Storage Iron (25-30%)			
1. Ferritin and haemosiderin	Liver, spleen and bone marrow	25 – 30	Yes, on demand

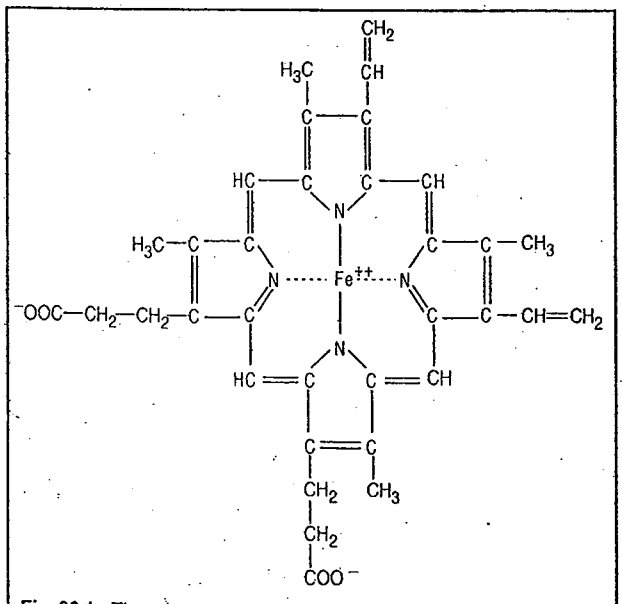


Fig. 36.1. The structure of haem.

Absorption, Storage and Metabolism (Iron Turnover; Ferrokinetics)

Iron in food is present in two forms - haem-iron and non-haem iron (inorganic iron). The haem-iron (mainly present in meat) is absorbed as such (intact iron) and is released in the mucosal cell by action of haem-oxidase enzyme. The non-haem iron is present mainly in ferric state that has low solubility and, therefore, poor absorption. In stomach, the ferric iron is reduced to the ferrous state by gastric acid that is more soluble and is more readily absorbed. Deficiency of gastric acid (e.g. achlorhydria), therefore, impairs iron absorption, whereas reducing agents like ascorbic acid favours iron absorption. Ascorbic acid stimulates iron absorption partly by forming soluble iron-ascorbate complex and partly by reducing ferric iron to ferrous iron.

Iron is absorbed mainly from the proximal jejunum and duodenum. A protein called divalent metal transporter which transports all kinds of divalent transports the iron across the into the cell. In the intestinal is re-oxidised to the insoluble

intracellular carrier protein apoferritin to form a complex called ferritin (Figure 36.2). The ferric iron is then either passed on to the plasma (if iron stores are low) or held in storage form in the mucosal cell (if body stores of iron are high). Ferroportin, a transmembrane iron channel protein, transports iron from the inside of the enterocytes in the duodenum to the outside of it. When transported out of mucosal cell into plasma, ferritin dissociates and the ferric iron combines with a glycoprotein beta1-globulin known as transferrin. In this bound form, iron is carried to storage depots (e.g. liver, spleen and bone marrow) and is transferred to cells via specific receptors which interact with transferrin. In the cells, iron again combines with apoferritin to become ferritin, the soluble and exchangeable storage form of iron. Small quantities of iron are also stored as the very insoluble haemosiderin. Stored iron can be re-utilised for erythropoiesis.

The excess iron in ferritin (storage iron) is lost into the lumen of gut and excreted in faeces. This mainly occurs by desquamation of ferritin bearing cells in the gut. Excess iron in circulation or storage cells is also excreted in bile and in traces in urine, nails and sweet.

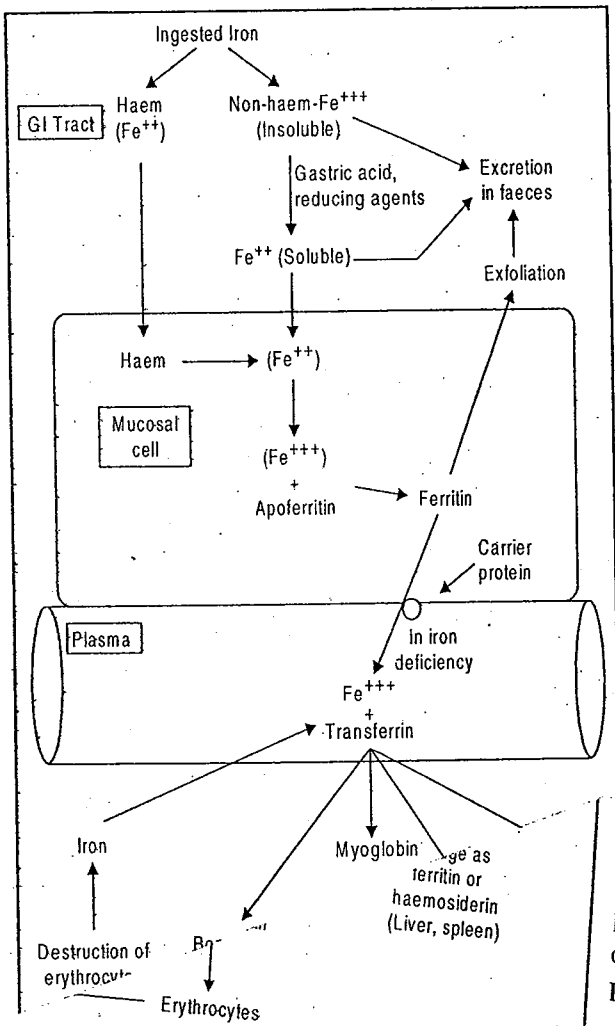


Fig. 36.2. Schematic representation of absorption, distribution storage and excretion of iron (see text for detail).

Cats

2 weeks or more.

Regulation of Iron

Iron is essentially needed in the body, so it is conserved and recycled in a highly efficient manner. Most of the iron released after destruction of erythrocytes is immediately reconverted to newly formed haemoglobin and only 10% of total body iron is lost per year. The total body iron is regulated primarily by absorptive mechanisms, which in turn depend on the body's iron stores. If the iron stores are less, then absorption of iron will be more. If the iron reserves are sufficient and all apoferritin in the intestinal mucosal cell has been combined with iron, then additional iron does not assimilate in mucosal cell and its absorption from diet will be less. This barrier of intestinal mucosal cell has been referred to as 'mucosal barrier or ferritin curtain', and the mechanism is called the 'mucosal block phenomenon'.

Recent research suggests that hepcidin, a peptide hormone secreted by liver, is the master regulator of iron metabolism in humans and animals. Hepcidin binds to the ferroportin, the iron channel protein present on enterocytes in the duodenum and blocks the iron transport from inside of enterocyte to the hepatic portal system thereby functionally reducing iron absorption.

Deficiency of Iron

Iron deficiency is the most common cause of nutritional anaemia in human beings and animals like pigs. Iron deficiency usually results from acute or chronic blood loss, from insufficient intake during periods of accelerated growth, or in pregnant animals. Severe iron deficiency results in characteristic microcytic hypochromic anaemia. Iron deficiency can affect metabolism in muscle independently of the effect of anaemia on oxygen delivery resulting in decreased activity of iron-dependent mitochondrial enzymes. Iron deficiency also has been associated with behavioural and learning problems and abnormalities in catecholamine metabolism and heat production.

Pharmacology of Iron

Iron is an essential mineral required for the transport of haemoglobin and myoglobin and utilisation of oxygen. Commercially iron is available in both oral and parenteral preparations. Oral preparations are available as ferrous (-sulphate, -fumarate, -succinate and -gluconate) and ferric (-ammonium citrate, -hydroxide, -choline citrate and -glycerophosphate) salts. Ferrous salts (mainly ferrous sulphate) tend to be inexpensive, are well absorbed. Parenteral preparations are available as iron-carbohydrate complexes (iron-dextran and iron-sorbitol citric acid) and are indicated when oral preparations cannot be tolerated or are not feasible. Parenteral iron may also be used in presence of severe iron deficiency or along with erythropoietin when absorption of oral iron may not be sufficient to meet the demand of induced rapid erythropoiesis. Parenteral preparations of iron may also be indicated in conditions like inflammatory bowel disease when iron absorption is impaired or in gastric upsets when iron absorption is insufficient.

Pharmacological effects

After oral or parenteral administration, iron is absorbed and efficiently utilised for the synthesis of haemoglobin. Within a week of iron therapy, there is brisk reticulocytosis indicating fresh erythropoiesis. The haemoglobin levels usually increase significantly within 2 to 4 weeks of oral therapy and reach normal level in 1-3 months. The response to iron-therapy, however, is

Table 36.3: Comparative features of iron-dextran and iron sorbitol citric acid.

Features	Iron-dextran	Iron sorbitol citric acid
Mol. Weight	High	Low
Route of administration	IM or IV	Only IM
Local binding after IM injection	10 to 30% gets locally bound which is not available for immediate utilization.	Not locally bound.
Mode of absorption	Given IM slowly absorbed through lymphatics.	Absorbed directly into circulation.
Binding to tranferrin	Does not bind.	Binds to transferrin.
Mode of distribution	Taken up by macrophages, slowly made available to erythron.	Directly available.
Excretion	Not excreted	30% excreated in urine.

influenced by several factors including the cause and severity of iron- deficient state, presence of other complicating illness, and ability to tolerate and absorb medicinal iron. Haemoglobin formation requires also adequate supply of pyridoxine and trace elements copper and cobalt (necessary for vitamin B12 synthesis by ruminal microflora). In general, utilization of iron for production of red cells is proportional to the severity of anaemia.

Pharmacokinetics

Oral preparations: The pharmacokinetics of oral preparations is similar to those of dietary iron as discussed earlier. The ferrous salts are more soluble in the gastric environment than ferric salts and are absorbed more rapidly. Variations in the particular ferrous salts have little effect on bioavailability as all ferrous salts are absorbed to approximately the same extent. Dietary sugars (fructose), several amino acids and some organic acids (e.g. citric acid, lactic acid and succinic acid) enhance the absorption of iron from GI tract, mainly by increasing its solubility. Pancreatic secretions, phosphates, oxalates, bicarbonates, etc. inhibit absorption of iron from the GI tract.

Parenteral preparations: Iron dextran (a colloidal solution containing 50 mg elemental iron/ml) is used by IM and IV routes. After IM administration, it is slowly absorbed via the lymphatic system. With IV administration, all iron is biologically available, but after IM administration a significant amount (up to 30%) of iron-dextran may remain bound to muscles at the site of injection for several months. After absorption, the reticuloendothelial cells of liver, spleen, and bone marrow and macrophages gradually clear iron-dextran from plasma. The iron is then cleaved from the carbohydrate (dextran) component. The iron is immediately bound to protein element to form ferritin, haemosiderin or transferrin and the dextran is either metabolised or excreted. Injected iron is not readily eliminated from body; only trace amounts of iron are excreted in the faeces, bile or urine. Iron also crosses placenta and may be excreted in milk in very small quantity.

Unlike iron-dextran, iron-sorbitol (iron-sorbitol citric acid complex) is rapidly absorbed from the injection site and is stable in tissue fluids. Two-thirds of the injected dose is absorbed from an IM site within 3 hours, most of it directly into the blood circulation and, to a lesser extent, via lymphatics. It is not bound locally and is not taken up by macrophages. After absorption, it binds to transferrin and then delivered to the target/storage site. The amount of iron sorbitol taken up by the liver and bone marrow is determined by iron status. A larger proportion is taken up by the liver in non-anaemic patients than in the iron deficient individuals. Iron-sorbitol is excreted in urine up to about 30%. Within approximately 10 days, little or no residue of iron remains at the injection site.

Side effects/Adverse effects

Oral iron may give rise to GI upsets like nausea, constipation or diarrhoea, and even abdominal cramps. Prolonged use of large doses of iron may lead to mucosal block of its absorption. Intolerance to oral preparations is some patients is primarily a function of the amount of soluble iron in the upper GI tract. Intramuscular administration of iron-dextran may give rise to local pain and brownish discolouration of tissue overlying the injection site. Occasionally, pigs may react to IM injection by prostration and muscular weakness. In case of IV therapy, there may be fever, malaise, urticaria, and rarely anaphylactoid reaction and death. Administration of iron sorbitol citric acid by IV route rapidly saturate the tranferrin and the excess iron can cause a toxic reaction i.e. hypotension, flushing, dizziness and vomiting. When iron sorbitol is given IM the iron release proceeds at a controlled rate, greatly reducing the potential for a toxic reaction.

Acute iron toxicity seen in humans and animals after ingestion of large quantities of iron salts may cause severe necrotising gastritis with vomiting, bloody diarrhoea and shock followed by circulatory collapse. Clinical signs of iron toxicosis in baby pigs may be expressed by pale skin, corrosion of mucosa, dark faeces, bloody diarrhoea, tachycardia, hypotension and shock.

Treatment: Iron toxicity may be treated by parenteral administration of deferoxamine (see for detail Chapter 76). Oral ascorbic acid administered concurrently with deferoxamine enhances excretion of iron.

Contraindications and precautions

Iron is contraindicated in patients with haemosiderosis, haemochromatosis, haemolytic anaemia, or known hypersensitivity to iron preparations. Oral iron preparations should not be used in patients with GI ulcerative diseases because of the GI irritating properties of the drug. Parenteral iron preparations should not be used in patients with acute renal infections. Parenteral and oral iron preparations should not be used in conjunction. In human patients, iron injection should be preceded by a test dose (about 0.5 ml) to avoid any systemic adverse reaction. In piglets, iron is always administered in the form of a complex (e.g. iron-dextran) in order to avoid the toxic effects caused by ions of free iron. Intramuscular iron injection should always be given in a large muscle mass. Iron sorbitol may form a grey precipitate when refrigerated and in this precipitated condition it should not be used.

Table 36.4: List of selected iron preparations.

Preparation	Iron contents (%)
I. Oral Preparations	
1. Ferrous sulphate	
Hydrated salt	20
Exsiccated salt	30
2. Ferrous fumarate	33
3. Ferrous gluconate	12
4. Ferrous succinate	
5. Ferrous aminoate	10
6. Ferric ammonium citrate	16-18
7. Ferric choline citrate (Iron choline citrate)	13-16
8. Colloidal ferrichydroxide	50
9. Ferric glycerophosphate	
II. Parenteral Preparations	
1. Iron-dextran (complex of ferric hydroxide and low molecular weight dextran)	5
2. Iron sorbitol (iron-sorbitol-citric acid complex)	6% (Dialyzable iron)

Drug interactions

Iron salts are chemically incompatible with many chemicals and drugs, so mixing of different drug preparations are not advised. Several drugs including tetracyclines and autacoids, and several foods (e.g. milk and egg) bind to and precipitate iron when given orally. In the gut, iron chelates with levodopa, carbidopa, penicillamine and ciprofloxacin and forms complexes with thyroxine, captopril and folic acid. Chloramphenicol may delay the response to iron administration by depressing bone marrow, so using chloramphenicol in patients with iron deficiency should be avoided. Allopurinol, a drug used to treat gout, can increase the amount of iron stored in the liver. Iron sorbitol should not be administered concurrently with oral iron medication.

Clinical uses

In veterinary medicine, indications of iron therapy are limited mainly to treatment or prevention of iron-deficiency that occurs with blood loss, pregnancy or successful therapy with anabolic steroids or recombinant erythropoietin in patients suffering from chronic anaemia. Injectable iron products are generally used in the treatment of iron deficiency anaemias associated with newborn animals (e.g. piglets). In piglets, iron supplements in the form of a complex such as iron-dextran are usually given in the first week of life. Because of the labour involved in multiple oral administrations, a single IM injection of iron-dextran on the 2nd or 3rd day of life is often given in newborn pigs.

Response to iron therapy can be assessed by monitoring circulating haemoglobin concentration. Iron preparations come as such or in combination with other haematinics and tonics. The efficacy of shotgun products (preparations containing a combination of haematinic agents), however, is questionable.

Dose

Ferrous sulphate

Dogs : 100 – 300 mg (total), PO, once daily for 2 weeks or more.

Cats : 50 – 100 mg (total), PO, once daily for 2 weeks or more.

Cattle : 8 – 15 g (total), PO, once daily for 2 weeks or more.

Horses : 2 – 8 g (total), PO, once daily for 2 weeks or more.

Sheep & swine : 0.5 – 2 g (total), PO, once daily for 2 weeks or more.

Iron-dextran

For treatment of iron-deficiency anaemia

Dogs : 10 – 20 mg/kg, IM, once daily followed by oral therapy with ferrous sulphate.

Piglets : 100 – 200 mg of elemental iron (total), IM. It may be repeated in 10 to 40 days.

For prevention of iron-deficiency anaemia in neonates

Kittens : 50 mg (total), IM, at 18 days of age.

Piglets (1-3 days) : 100 – 150 mg of elemental iron (total), IM

Cobalt

Cobalt is a chemical element with symbol Co and atomic number 27. It is an essential trace element and a key component of cobalamin, also known as vitamin B_{12}. Vitamin B_{12} is synthesised by the rumen microflora and, therefore, cobalt supplementations are necessary for erythropoiesis in ruminants. Non-ruminants do not require elemental cobalt in their diets because they receive vitamin B_{12} preformed (see for detail Chapter 55).

Mechanism of Action

Cobalt as a component of vitamin B_{12} is necessary for erythropoiesis in ruminants. It enhances synthesis of vitamin B_{12} by rumen microflora in the ruminants and may also increase production of erythropoietin in the kidneys. Therefore, cobalt indirectly stimulates stem cell division and red cell maturation and release. Cobalt also facilitates the incorporation of iron into haem. Cobalt through vitamin B_{12} also facilitates conversion of propionic acid, a major glucogenic rumen fermentation metabolite, to glucose in the liver.

Deficiency of Cobalt

Deficiency of cobalt results in functional deficiency of vitamin B_{12}. In ruminants, Co deficiency is associated with marked anaemia, decrease in blood volume, decreased appetite, loss of body weight, emaciation, and failure to thrive. There is non-specific reduction in immunity in sheep and an associated increased prevalence of parasitic and bacterial infections. Cobalt deficiency predominantly affects young growing ruminants and is more severe in sheep than the cattle. Cobalt deficiency is more common on pasture, particularly in regions where soil is deficient in cobalt.

Pharmacology of cobalt

Cobalt is used clinically for both preventive (mainly) and therapeutic measures. Cobalt is available commercially as cobalt-sulphate, -oxide and –chloride salts. Deficiency of cobalt can be prevented by oral administration of a slow release ruminal bolus/pellet containing cobalt oxide. This cobalt oxide bolus/pellet remains in the reticulum and releases cobalt slowly over a long period. Cobalt preparations combined with iron are also being used in therapy of certain anaemias and as general tonics. Cobalt salts are sometimes included in the trace mineral premixes and salt blocks, or may be incorporated as a component of feed concentrates. In endemic deficiency areas, top dressing of the soil with 100 – 150 g (or even higher) cobalt sulphate per acre is generally used to prevent deficiency.

Cobalt salts are not effective parenterally because it is required for synthesise of vitamin B_{12} which is manufactured only in the rumen by ruminal microflora. Injection of cyanocobalamin is more useful for the treatment of the effects of cobalt deficiency in ruminants. Excess cobalt has been associated with damage to pancreatic alpha (glucagons producing) cells and polycythemia. Administration of ruminal blouses to animals less than 8 week of age is not recommended.

Dose

Cobalt salts

For treatment of cobalt deficiency

Cattle : 0.5 g (total), PO.
Calves : 0.2 g (total), PO.
Sheep : 0.1 g (total), PO.
Lambs : 0.05 g (total), PO.

For prophylaxis of cobalt deficiency

Cattle : 25 mg (total), PO, once daily either in feed or as a drench.
Calves : 10 mg (total), PO, once daily.
Sheep : 5 mg (total), PO, once daily in feed.
Lambs : 2.5 mg (total), PO, once daily.

Cobalt oxide ruminal bolus/pellet

For prophylaxis of cobalt deficiency

Cattle : One 9 g ruminal bolus.
Sheep : One 3 g ruminal bolus.

Copper

Copper is a chemical element with the symbol Cu (Latin: *cuprum*) and atomic number 29. It is an essential component of several important enzymes and is required for the formation of haemoglobin, bone, melanin and keratin. Copper deficiency is rare in human beings, but may be seen in animals.

Mechanism of Action

Copper is an integral component of some important enzymes including ascorbic acid oxidase, polyphenol oxidase, cytochrome oxidase and monoamine oxidase. As a haematinic, it is necessary for normal utilisation of iron in haemoglobin, and helps in iron-absorption and in increasing outflow of iron from reticulo-endothelial cells. The deficiency of copper leads to microcytic anaemia related to both a decrease in availability and to a decrease in mitochondrial production of haem.

Deficiency of Copper

Deficiency of copper is common in young growing ruminants in certain geographical areas. Primary deficiency may occur as a result of inadequate dietary intake of copper, whereas secondary copper deficiency occurs because of high levels of molybdenum, iron, or sulphur in the diet. In growing animals, primary and secondary copper deficiency can cause unthriftiness, loss of coat colour, anaemia, diarrhoea and temporary sterility. If copper deficiency is prolonged, the structure of collagen is altered to produce deformity of long bones.

Pharmacology of Copper

Copper is used clinically for both preventive and therapeutic purposes. Commercially, copper salts are available for both parenteral and oral administration. Parenteral copper salts include calcium copper edetate, cuproxoline, copper glycinate, copper methionate (copper methionine) and copper-heptonate. Preparations based on methionine or heptonate complexes are slowly mobilised from the injection site and, therefore, are used as depot preparations. Intravenous administration is also used occasionally in cattle and sheep. Oral administration of copper salts (e.g. copper sulphate) can also correct copper deficiency, but more slowly than parenteral treatment because of the time taken for intestinal absorption and possible inhibition by dietary sulphate, iron and molybdenum. Copper-oxide preparations are available which provide depot effect from the oral route. Top dressing of pastures with copper sulphate is practised in some countries like Australia and New Zealand. In deficient areas, copper in the form of cupric sulphate is also added to salt at a rate of 0.5%.

Copper in excess is toxic, so its clinical use in animals should be done carefully. Sheep are more prone to poisoning by copper hence copper salts such as calcium copper edetate and cuproxoline, which are rapidly mobilised from injection site, should not be used in sheep. Copper preparations should not be used in patients with hepatic or renal impairment.

Dose

For treatment of copper deficiency

Copper sulphate

Cattle (adult) : 1 – 2 g, PO, once daily for 7 days, then a break of 5 days, and again treatment for 7 days.
Young cattle : 0.5 – 1 g, PO, once daily for 7 days, then a break of 5 days, and again treatment for 7 days.
Sheep : 0.25 g/45 kg, PO, once daily.

Copper glycinate (Depot preparation)

Cattle : 120 mg, IM.
Sheep : 40 mg, IM.

Calcium copper edetate

Cattle (< 18 months of age) : 50 – 100 mg, SC.
(> 18 months of age) : 100 – 200 mg, SC.

Copper heptonate

Sheep : 25 mg (total), IM, usually given 10 weeks before lambing.

For prevention of copper deficiency

Pasture dressing

Copper sulphate : 4 kg/hectare/year.

Mineral licks

Copper sulphate : 0.5% of salt.

2. VITAMINS

Vitamin B_{12}

Vitamin B_{12} (Cobalamin) is a water soluble vitamin that is essentially required for DNA synthesis and cell proliferation. Since the erythropoietic centres of bone marrow are among the most rapidly growing and proliferating tissues, inadequate supply of vitamin B_{12} often results in reduced RBC proliferation and a 'maturation arrest' in the bone marrow. The erythroblast cannot continue to divide, becomes very large and is referred to as a megaloblast. Similarly, the mature erythrocyte is also large and is referred to as a macrocyte. Although the oxygen-carrying capacity of macrocyte is adequate, the enlarged cell is very fragile and has a reduced life span. In addition, the total red cell count is often very low. The clinical manifestation of such deficiency is megaloblastic or pernicious anaemia.

Naturally occurring anaemia from deficiency of vitamin B_{12} is infrequently recognised in domestic animals. A dietary shortage of cobalt, which is required for ruminal organisms in synthesis of

vitamin B_{12}, can result in an indirect deficiency of the vitamin in ruminants. In humans, however, vitamin B_{12} deficiencies can develop secondarily to either inadequate dietary supplies or inadequate absorption of the vitamin from GI tract. The pharmacology and clinical uses of vitamin B_{12} are discussed in Chapter 55.

Folic acid

Folic acid, like vitamin B_{12}, is required for synthesis of nucleoproteins involved in red cell maturation. It is a cofactor needed for DNA synthesis and is also required for RNA synthesis and formation of vitamin B_{12}. Anaemias associated with a lack of folic acid are characterised as megaloblastic and macrocytic. Anaemia from folic acid deficiency occurs rarely in most animal species. The pharmacology and clinical uses of folic acid are described in detail in Chapter 55.

Pyridoxine (Vitamin B_6)

Pyridoxine (Vitamin B_6) promotes the red blood cell production and is required for the biosynthesis of monoamine neurotransmitters serotonin, dopamine and norepinephrine. However, pyridoxine responsive anaemia is not a common entity in human beings and animals. In some species such as dogs and pigs, an abnormality in haem synthesis resulting in microcytic and hypochromic anaemias responds to pyridoxine therapy. Routine use of pyridoxine in anaemia is not advised (see for detail Chapter 55).

Riboflavin (Vitamin B_2)

Riboflavin, commonly called vitamin B_2, is required for a wide variety of cellular processes. A type of hypoplastic anaemia occurs in riboflavin deficiency in human beings, which is generally considered a part of multiple deficiencies in protein-calorie malnutrition. In the absence of specific deficiency, use of riboflavin in anaemia is of no value (see also Chapter 55).

Ascorbic acid (Vitamin C)

Ascorbic acid (Vitamin C) is a water-soluble vitamin that under normal condition is synthesised by all species of animals. Guinea pigs, monkeys and man, however, cannot synthesise the vitamin and, therefore, require it as an essential dietary supplement. In addition to its involvement in various metabolic and immune functions (see for detail Chapter 55), ascorbic acid facilitates iron absorption.

II. HAEMATOPOIETIC GROWTH FACTORS

The haematopoietic growth factors are a family of cytokines or glycoprotein hormones which regulate the proliferation and differentiation of haematopoietic progenitor cells in the bone marrow. Some of these factors are multikinetic, exerting their effects on multiple cell lineages, whereas others are cell lineage specific. These factors include erythropoietin, thrombopoietin, stem cell factor (SCF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukins. Many of these growth factors have been identified, purified, cloned and extensively studied. Some of these have been produced by recombinant DNA technology and are available for clinical use.

Physiology

The erythrocytes, granulocytes, mononuclear cells and platelets are all physiologically derived from a relatively small number of self-renewing pluripotential haematopoietic stem cells (HSCs) in the bone marrow. As these pluripotential stem cells reproduce, continuing throughout the life, a small portion of them remains exactly like the original cells and are retained in the bone marrow to maintain the supply. The larger portion of the reproduced stem cells, however, differentiates to form progenitor

Table 36.5: Important functions of some selected haematopoietic growth factors.

Sr. No.	Haematopoietic Growth Factor	Abbreviation	Primary Function
1.	Stem cell factor	SCF	Stimulates early pluripotential and committed progenitor cells.
2.	Erythropoietin	EPO	Stimulates proliferation and maturation of committed erythroid progenitor cell (CFU-E/BFU-E) to enhance erythropoiesis.
3.	Thrombopoietin	TPO	Stimulates proliferation and differentiation of haematopoietic progenitor cell (CFU-Meg) to enhance thrombopoiesis.
4.	Granulocyte-mactrophage colony stimulating factor	GM-SCF	Stimulates CFU-G, CFU-M, and CFU-Meg to increase granulocytes and monocytes production. It may also promote CFU-E/BFU-E formation through EPO.
5.	Granulocyte colony stimulating factor	G-CSF	Stimulates CFU-G to increase granulocytes production. Also enhances phagocytic and cytotoxic activity of neutrophils.
6.	Macrophage colony stimulating factor	M-CSF, CSF-1	Stimulates CFU-M to increase monocytes production. Also enhances function of monocytes/macrophages.
7.	Interleukins	IL	
	a. Il-1, IL-3, IL-5, IL-6, IL-9 and IL-11		Act synergistically with each other and SCF, GM-CSF, G-CSF and EPO to stimulate CFU-E/BFU-E, CFU-G, CFU-GM, CFU-Meg growth. Also have numerous immunological roles, including mast B cell and T-cell growth stimulation.
	b. IL-5		Controls eosinophils survival and differentiation.
	c. IL-1, IL-2, IL-4, IL-5, IL-7 and IL-12		Stimulates growth and function of T-cells, B-cells, NK cells, and monocytes.
	d. IL-8		Acts as chemotactic factor for basophils and neutrophils.

Note: For detailed action of interleukins, see also Chapter 56.

Abbreviations: BFU-E = burst forming unit erythrocyte, CFU-E = colony forming unit erythrocyte; CFU-G = colony stimulating unit granulocyte, CFU-GM = colony forming unit granulocyte/macrophage; CFU-M = colony forming unit macrophage; CFU-Meg = colony forming unit megakaryocyte; NK = natural killer.

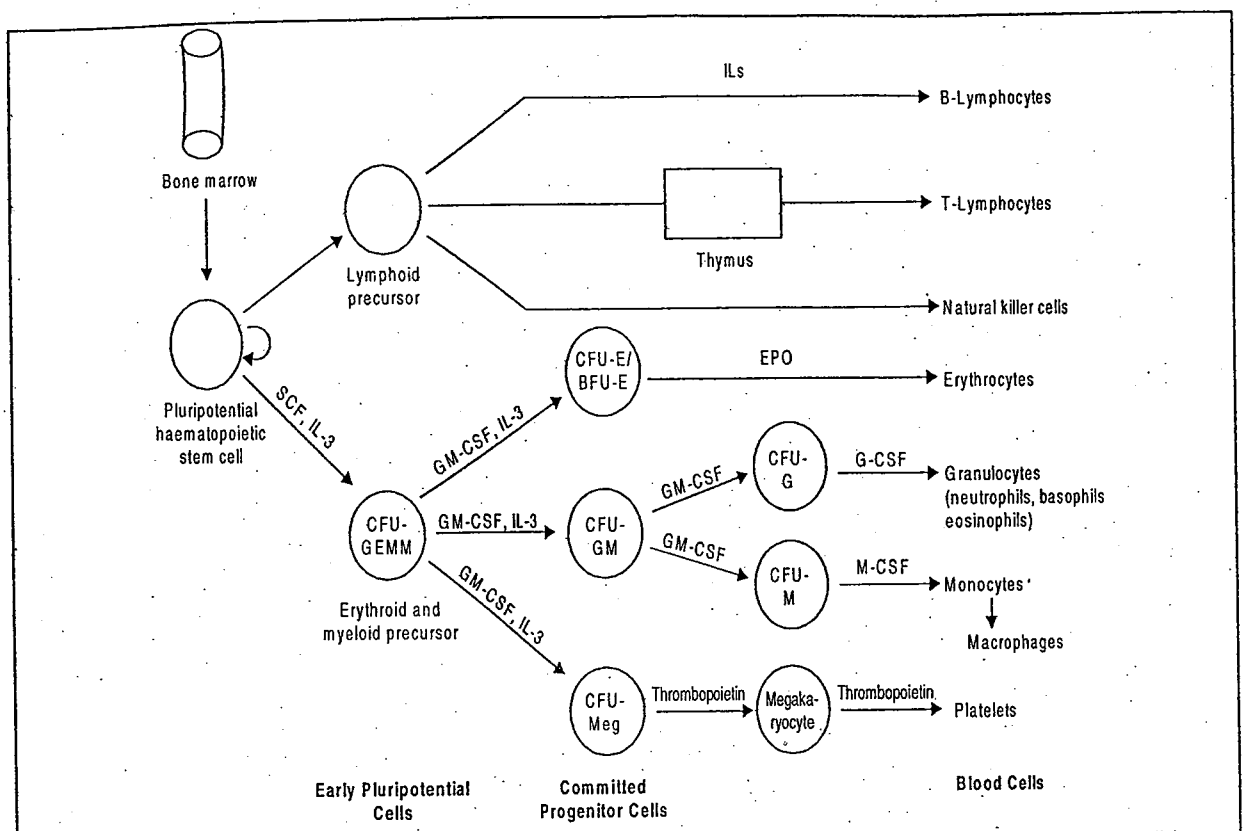


Fig. 36.3. Schematic representation of site of action of haematopoietic growth factors in the differentiation of blood cells (see text for details).
Abbreviations : BFU-E = burst forming unit-erythrocyte ; CFU-E = colony forming unit - erythrocyte ; CFU-G = colony forming unit - granulocyte ; CFU-GEMM = colony forming unit - granulocyte / erythrocyte/macrophage/megakaryocyte ; CFU - GM = colony forming unit granulocyte/macrophage; CFU-M = colony forming unit - macrophage ; CFU - Meg = colony forming unit megakaryocyte ; EPO = erythropoietin ; G-CSF = granulocyte colony stimulating factor ; GM-CSF = granulocyte/macrophage colony stimulating factor ; ILs = interleukins ; IL-3= interleukin 3; M-CSF = macrophage colony stimulating factor ; SCF = stem cell factor.

cells under the influence of some cellular and humoral factors (also called growth factors) viz. stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF) (Table 36.5). Although the early progenitor cells (also called burst-forming units [BFU] and colony forming units [CFU]) still cannot be recognised as different from the pluripotential stem cells, they become committed to a particular line of cells and hence are called committed progenitor cells or committed stem cells. The different committed progenitor cells, when grown in culture, produce colonies of specific types of blood cells (hence called colony stimulating unit).

A committed progenitor that forms erythrocytes is called a colony forming unit-erythrocyte (CFU-E) and which forms granulocytes and monocytes is called colony forming unit granulocyte monocyte (CFU-GM), and so forth (Figure 36.3). The proliferation and differentiation of the progenitor for each cell line into blood cells is regulated by an overlapping set of growth factors (e.g. erythropoietin, granulocyte-colony stimulating factor [G-CSF], monocyte/macrophage colony stimulating factor [M-CSF or CSF-1], thrombopoietin, etc.). Erythropoietin is required for a myeloid progenitor cell to become an erythrocyte, and thrombopoietin is required for a myeloid progenitor cell to differentiate to megakaryocytes and thereafter thrombocytes (platelets). The haematopoietic and lymphopoietic growth factors are produced by a number of marrow cells and peripheral tissues. These growth factors initiate signal transduction pathways and alter transcription factors, which in turn activate genes which determine the differentiation of blood cells. Most of these growth factors show synergistic interaction with other factors, as well as 'networking', wherein stimulation of a cell lineage by one growth factor induces the production of additional growth factor.

1. Erythroid Growth Factors

Erythropoietin

Erythropoietin (Epoetin; EPO) is an endogenous glycoprotein hormone that regulates erythropoiesis. The kidney (peritubular cells) is the primary source of endogenous erythropoietin, although the liver produces also a small amount of EPO in some species. In bone marrow, it is also produced by macrophages. Physiologically, its level is regulated by haemoglobin concentration, artificial oxygen saturation, or both (Erythropoietin production and release from kidney are stimulated by anaemia and hypoxia). The gene for erythropoietin has been cloned and two forms of recombinant human erythropoietin namely epoetin alpha and epoetin beta are prepared. Commercially, recombinant human erythropoietin (rhEPO) is available mainly as epoetin alpha for parenteral administration. Epoetin beta is available also and has clinical efficacy similar to that of epoetin alpha. Canine erythropoietin has also been isolated and synthesised in recombinant form, but is not yet commercially available.

Chemistry

Erythropoietin is a sialoglycoprotein hormone produced by peritubular cells of kidneys (so chronic renal failure is associated with anaemia). Human erythropoietin has a molecular weight of 304,000. Substantial differences exist between the human and canine

proteins. Glycosylation is important for prolonging the half-life of erythropoietin in circulation, but not for biological activity.

Mechanism of action

Exogenous administration of recombinant human erythropoietin (rhEPO) serves as a substitute for endogenous erythropoietin. Its influence on erythropoiesis occurs at several steps. It binds to specific erythropoietin receptor (EpoR) on the surface of erythrocytes and erythroid precursor cells and initiates changes in the intracellular phosphorylation. This receptor is also found in a large number of tissues such peripheral/central nerve cells, many of which activate intracellular biological pathways upon binding with EPO.

Erythropoietin stimulates the proliferation and differentiation of erythroid precursors including colony-forming unit erythrocytes (CFU-E) from haematopoietic stem cells in the bone marrow, and also pro-erythroblasts, erythroblasts and reticulocytes. Once the pro-erythroblasts are formed from stem cells, the erythropoietin also causes these cells to pass more rapidly through different erythroblastic stages than normally further speeding up the production of new cells. Erythropoietin also induces haemoglobin synthesis and erythroblast maturation. In addition, erythropoietin stimulates the release of reticulocytes from bone marrow into blood, where they subsequently mature. Erythropoietin also has significant effect on red blood cells by promoting their survival through decreasing apoptosis. In addition to its effects on erythropoiesis and erythrocytes, erythropoietin performs other functions as well including role in wound healing and brain's response to neuronal injury.

Pharmacological effects

Parenteral administration of human recombinant erythropoietin (rhEPO) stimulates production of red cells. It acts on the marrow in concert with other humoral mediators to increase the number of stem cells entering RBC production. As final synthesis of red cells involves several intermediately stages, new erythrocytes appear in the circulating blood only after 5 days. Response to epoetin is indicated by reticulocytosis and an increase in the haematocrit. The haematocrit, haemoglobin concentration and RBC count normalise within 3 to 4 weeks of therapy in anaemia patients, while whole blood cells and platelets do not seem to be affected.

Erythropoietin has a range of other actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It has also been shown that erythropoietin can increase iron absorption by suppressing the hormone hepcidin.

Pharmacokinetics

The pharmacokinetics of rhEPO has not been well characterised in either humans or animals. It is absorbed only after parenteral administration. The drug's metabolic fate in not clear. In humans, the elimination half-life is about 6 to 9 hours with a very small volume of distribution (0.055 L/kg). In patients with chronic renal failure, half-life is prolonged approximately 20% over those with normal function. As with most endocrine products, plasma half-life may not necessarily reflect biological half-life.

Side effects/Adverse effects

The main adverse effect of rhEPO in animals (up to 30%) is development of antibodies with resultant resistance to further treatment. Both exogenous and native erythropoietin may be impaired due to development of antibodies within 2 weeks, which may be accompanied by a progressive decrease in RBCs, haematocrit and haemoglobin and ultimately aplastic anaemia due to bone marrow failure. A myeloid : erythroid ratio of greater than 6 predicts significant autoantibody formation and contraindicates further EPO therapy. Discontinuation of rhEPO will result in resolution of antibody formation and to some extent also anaemia, but drug therapy cannot be reconstituted.

Other adverse effects which may require monitoring in patients receiving rhEPO include systemic hypertension, iron deficiency, hyperkalaemia and polycythemia. Local and systemic allergic reactions (which may be predictors of antibody formation), fever, arthralgia and mucocutaneous ulcerations have been reported in some animals.

Contraindications and precautions

Epoetin is contraindicated in patients those are hypersensitive to it or with uncontrolled hypertension. Its use in pregnancy is advised only when benefits outweigh the potential risks. Regular haematological monitoring is required to ensure that the packed cell volume does not rise above normal. Informed consent should be obtained from owner of animal before EPO use because of the high incidence of antibody formation. Iron deficiency before and during therapy may blunt the response to EPO because stored iron in body may not be sufficient to support increased erythropoiesis. The dosage should not be adjusted more often than once every week because of the long lag time for a response. If the adequate response is not achieved with EPO, the patient should be carefully revaluated for other diseases like iron deficiency, haemolysis, infections and inflammatory conditions

Drug interactions

Anabolic or androgenic steroids (e.g. nandrolone) may potentially increase the response to EPO. Probenecid has been shown to reduce the renal tubular excretion of EPO.

Clinical uses

The primary indication for EPO therapy in human and veterinary medicine is anaemia with chronic renal failure. When administered in uremic animals with chronic renal disease, the haematocrit of most patients normalises within 3 to 4 weeks of therapy, and the clinical well being improves. It may also be considered useful in anaemias resulting from myelosuppression caused by antineoplastic drugs. Anaemias not related to renal disease (e.g. inflammatory disease, neoplasm and haemolytic disease) do not respond to epoetin therapy. Because of high cost and potential risks associated with its use, epoetin should only be considered when the packed cell volume in dogs or cats is 20% or less due to renal failure.

Epoetin has also proved useful in some cases of non-renal disease anaemia in humans like sickle cell anaemia and multiple myeloma. Most human patients receiving renal dialysis also receive epoetin. Epoetin beta is also available and can be used clinically in place of epoetin alpha.

Dose

Epoetin alpha

Dogs & cats : 50 – 100 units/kg, SC, 3 times weekly until PCV is within normal range. Then dose is reduced to lowest effective level.

Darbepoetin alpha

Darbepoetin alpha is a synthetic form of erythropoietin that contains erythropoiesis-stimulating 165-amino acid protein. It differs from endogenous erythropoietin (EPO) by containing two more

N-linked oligosaccharide chains and is produced by recombinant DNA technology in modified Chinese hamster ovary cells. Similar to erythropoietin alpha, it stimulates erythropoiesis and is primarily used to treat anaemia, commonly associated with chronic renal failure and cancer chemotherapy. It is administered by intravenous or subcutaneous injection. Its adverse effect profile is similar to that of erythropoietin. Its use has been associated with increased risk of cardiac arrest, arrhythmia, hypertension and hypertensive encephalopathy, congestive heart failure, vascular thrombosis or ischemia, myocardial infarction and oedema.

2. Myeloid Growth Factors

Myeloid growth factors are glycoproteins which stimulate the proliferation and differentiation of one or more myeloid cell lines and also enhance the function of mature granulocytes and monocytes. Recombinant forms of several myeloid growth factors have been produced recently including granulocyte/macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte/macrophage colony stimulating factor (M-CSF or CSF-1), stem-cell factor (SCF), interleukin-3 (IL-3) and thrombopoietin. Some of these growth factors (e.g. G-CSF and GM-CSF) are available commercially in human medicine for therapeutic stimulation of granulopoiesis (proliferation, differentiation and function of granulocytes, macrophages and eosinophils). Both recombinant canine G-CSF (rcGSF) and recombinant feline G-CSF (rfGSF) have been cloned, but neither is commercially available at present. These factors are increasingly being studied for treatment of neutrophil disorders in dogs and cats.

a. Granulocyte-Colony Stimulating Factor (G-CSF)

Filgrastim

Filgrastim (Granulocyte-Colony stimulating Factor, G-CSF) is a human granulocyte stimulating factor and a biological response modifier. It is manufactured by recombinant DNA technology using *E. coli* as the host organism. Filgrastim as a growth factor stimulates the production, maturation and activation of neutrophils. It also stimulates the release of neutrophils from the bone marrow. In patients receiving chemotherapy, filgrastim can accelerate the recovery of neutrophils, reducing the neutropenic phase.

Filgrastim is used to stimulate the production of granulocytes in patients undergoing antineoplastic therapy. It is also used to prevent infection and neutropenic fevers caused by chemotherapy. In addition, filgrastim is indicated to increase neutrophil production in endogenous myeloid disorders, such as congenital neutropenic states. It is also considered during bone marrow transplantation therapy. Side effects are often predictable in terms of their onset and duration and may include pain in bones, irritation and itching at injection, fever, nausea and vomiting, diarrhoea, tachycardia, bleeding, rashes and abnormalities in blood count. Filgrastim should not be used along with aspirin or like-drugs. Filgrastim may be given by subcutaneous or intravenous injection. It has been approved for use in human medicine.

Pegfilgrastim

Pegfilgrastim (Pegylated G-CSF) is a PEGylated (covalent attachment of polyethylene glycol polymer chains) form of the filgrastim. The covalent attachment of a 20 kDa polyethylene glycol (PEG) molecule to the N-terminus of the filgrastim protein protects the parent drug from the host immune system (reduced immunogenicity and antigenicity) and increases its circulatory time by reducing renal clearance. Accordingly, pegfilgrastim has a human half-life of 15 to 80 hours, much longer than the parent filgrastim (3-4 hours). Pegfilgrastim treatment can also be used to stimulate the bone marrow to produce more neutrophils to fight infection in patients undergoing chemotherapy. Pegfilgrastim is approved primarily to reduce febrile neutropenia and is considered to be more effective than G-CSF in this regard. It is equally efficacious as filgrastim, with the advantage of once-per-cycle dosing and self-regulating features of clearance of the drug during neutrophil recovery.

Lenograstim

Lenograstim is a Chinese hamster ovary-derived recombinant human G-CSF consisting of 174 amino acids with 4% carbohydrate. It is more potent and stable than filgrastim and is primarily used as an immunostimulator. It has been investigated as a possible adjunctive treatment for severe sepsis and found to be ineffective.

b. Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF)

Sargramostim

Sargramostim is a recombinant myeloid lineage-specific granulocyte macrophage colony-stimulating factor (GM-CSF) produced in yeast. It is a glycoprotein of 127 amino acids and differs from the natural human GM-CSF by a substitution of leucine at position 23, and the carbohydrate moiety may be different from the native protein. On administration, sargramostim stimulates the production of neutrophils, monocytes and eosinophils. Sargramostim is primarily used for myeloid reconstitution after autologous or allogeneic bone marrow transplantation in human patients. It is also used to treat neutropenia induced by chemotherapy during the treatment of acute myeloid leukemia. It is also considered an immunostimulator. Sargramostim is generally well tolerated, but incidence of arrhythmia, fainting, eosinophilia, dizziness, hypotension, injection site reactions, pain tachycardia, thrombosis, and transient liver function abnormalities have been reported.

Molgramostim

Molgramostim is recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) containing 127 amino acids. It is produced by a strain of *Escherichia coli* (*E.coli*) bearing a genetically engineered plasmid containing the human GM-CSF gene. It primarily functions as a immunostimulator. The most frequently reported undesirable effects are fever, nausea, dyspnoea, diarrhoea, rash, rigors, injection site reaction, vomiting, fatigue, anorexia and musculoskeletal disorders. Molgramostim is contraindicated in patients with a history of hypersensitivity to molgramostim or any component of the injectable formulation. Molgramostim is not recommended in patients with myeloid malignancies.

c. Stem Cell Factor (SCF)

Ancestim

Ancestim is a recombinant methionyl human stem cell factor, commonly known by the trade name StemGen. It is produced in *E. coli* and contains 166 amino acids in a sequence identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine. Unlike natural CSF, ancestim is nonglycosylated. Ancestim is

indicated for use in combination with filgrastim in the setting of autologous peripheral blood progenitor cell (PBPC) transplantation. Its use is contraindicated in patients with known hypersensitivity to *E.coli*-derived proteins, ancestim or any component of the product. Ancestim should only be administered by SC injection and is approved for use in human medicine.

d. Interleukins

Oprelvekin

Oprelvekin is recombinant interleukin eleven (IL-11) produced in *Escherichia coli*. The polypeptide is 177 amino acids in length in contrast to 178 amino acids in the natural IL-11 and it is non-glycosylated. Oprelvekin functions as a thrombopoietic growth factor and directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces megakaryocyte maturation resulting in increased platelet production. Oprelvekin is indicated for the prevention of severe thrombocytopenia and the reduction of the need for platelet transfusions following myelosuppressive chemotherapy in human patients with nonmyeloid malignancies.

III. MISCELLANEOUS DRUGS

1. Anabolic or Androgenic Steroids

Anabolic steroids are synthetic compounds which are structurally related to testosterone. In addition to their protein-anabolic activity, anabolic steroids stimulate erythropoiesis. The proposed mechanisms include stimulation of erythropoietin production via EPO-stimulating factor, differentiation of stem cells into erythroid precursor cells and direct stimulation of erythroid-progenitor cells. Anabolic steroids also increase intracellular concentrations of 2, 3-bisphosphoglycerate in erythrocytes thereby enhancing oxygen release into tissues. Efficacy of anabolic steroids on the RBC mass depends on the presence of supportive material (e.g. sufficient bone marrow cellularity).

In veterinary medicine, anabolic steroids are used to stimulate erythropoiesis in some cases of anaemia, particularly those associated with renal disease accompanied by low EPO levels. The pharmacology and clinical uses of anabolic steroids have been described in Chapter 51.

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CHAPTER 37 BLOOD, BLOOD COMPONENTS AND BLOOD SUBSTITUTES

Whole blood/Blood
- Blood groups
- Donors
- Collection of blood
- Transfusion and pharmacology of whole blood

Blood components
- Packed red blood cells
- Plasma
- White blood cells
- Other blood components

Blood and plasma substitutes
- Blood substitutes
- Plasma substitutes/Plasma volume expanders

Blood is vital for the maintenance of life. In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral ions, hormones, oxygen, carbon dioxide and blood cells. Blood supplies cells with water, electrolytes, nutrients and hormones and removes waste products. The cellular elements supply oxygen (RBCs), protects against foreign organisms and antigens (WBCs) and initiate coagulation (platelets). The plasma proteins provide colloid osmotic pressure in the plasma, which in turn prevents plasma loss from the capillaries. Therefore, inadequacy of blood or blood components often result in improper functioning of various body processes which, if not corrected, may lead to serious complications like shock and kidney failure. Therapeutic use of blood or its constituents occupies very important place in human and veterinary therapeutics.

WHOLE BLOOD/BLOOD

Whole blood is a term used in medicine for the animal blood from which no constituent, such as red blood cells, white blood cells, plasma or platelets, has been removed. It is collected from a donor in some anticoagulant and is used for blood transfusion. If used immediately after collection, the whole blood (called fresh whole blood) contains all constituents of circulating blood including the coagulation proteins. On storage for specified period, some blood constituents such as coagulation proteins and enzymes may be lost. Whole blood may be purchased commercially or prepared in the clinics/hospital. Whole blood transfusion is indicated mainly in cases of acute blood loss, as in acute haemorrhage and haemolysis. It is also indicated in the treatment of acute or chronic anaemia, coagulation abnormalities and deficiencies of specific and non-specific antibodies.

Blood for transfusion is obtained from donors by blood donation and stored in a blood bank. Whole blood for transfusion can also be prepared in the clinics or hospital after careful matching of blood groups. Transfusion of blood of an incompatible blood group may cause severe, often fatal, complications, so cross-matching is done to ensure that a compatible blood product is transfused.

Blood groups

A blood group (also called a blood type) is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs), which determine the immunological specificity of the cell. These antigens may be proteins, carbohydrates, glycoproteins or glycolipids, depending on the blood group system. The number of major recognised blood group systems varies among domestic species with dogs, cattle and horses being the most complex and cats the most simple. Although canine blood has been characterised by at least eight groups (designated as dog erythrocyte antigen or DEA,), only three (DEA 1.1, 1.2, and 7) are reported to have significant clinical significance (Table 37.1). In horses, over 30 blood groups have been identified but only 8 are recognized as major systems, out of which again three are of more clinical significance. Animal blood groups are typed to aid in the matching of donors and recipients and also to identify breeding pairs potentially at risk of causing haemolytic diseases in their offspring. The incompatibility chances between donor and recipient are less on the first blood transfusion within 24 hours, but after this cross-matching is essential due to risk of antibodies formed against foreign protein causing a transfusion reaction.

Table 37.1: Major blood groups in animals.

Species	Number of recognised blood group systems	Blood groups of clinical significance
Dogs	8	DEA 1.1, DEA 1.2 and DEA 7
Cats	3	A and B
Cattle	12	B and J
Horses	8	A, C and Q
Sheep	7	B and R

Donors

Donor of blood should be young (3-6 years of age), healthy and cooperative with no history of blood transfusion. Dogs preferably should be DEA-1 and DEA-7 negative. In small animals, female donors should not have puppies or kittens and preferably should have been neutered. All donor animals should be free from any apparent acute or chronic infectious disease. Routine care for donor animals should include vaccinations, and internal and external parasiticide treatment. Records should be kept on donors, particularly with regard to date and amount of collections.

Collection of blood

Blood is collected in an anticoagulant in perfectly clean and sterile equipment. Two types of containers are available for collection of blood - vacuum bottles and plastic bags. Vacuum bottles are simple to use, but they may cause activation of platelets and some coagulation factors, increase chances of contamination, and also chances of breakage of bottle and potential for air embolism. In contrast, plastic bags remain sterile and activation of blood components is not as likely as with glass. However, collection of blood is more difficult into plastic bags compared to glass bottle as a positive (i.e. arterial) and negative (i.e. a syringe or a vacuum device) pressure is needed to generate blood flow sufficiently rapid to prevent clotting in the bag. Anticoagulants used for blood collection include acid citrate dextrose (ACD), citrate phosphate dextrose (CPD) and sodium citrate. Maintenance of normal RBC physiology is an important consideration in the selection of most appropriate anticoagulant.

Blood is generally collected from jugular vein in all animals; in dogs and cat it may require suction or vacuum. In dogs femoral artery can also be used for plastic bag collection, but it requires moderate sedation and added care to avoid haemorrhage. Blood may be collected by gravity flow from the jugular vein through a 10-gauge needle in larger species. Up to 20% of a donor's body weight (10 to 15 ml/kg) can be collected safely at 2 to 4 week intervals, but no more than 25% of a donor animal's blood should be collected at one time. Blood should be gently mixed throughout collection to equally dispose the anticoagulant. Collected blood should be either used within 24 hours or stored at refrigerator temperature (1 to 6° C) for subsequent use. Depending on the type of anticoagulant, the whole blood can be stored for up to 2 to 4 weeks.

Transfusion and Pharmacology of Whole blood

Blood transfusion into the recipient animal is always done aseptically and only by intravenous route. This requires sterile preparations of site and appropriate blood transfusion set. Filter should be used to remove clots and large particles. A large-gauge IV catheter should be used (20 gauge for dogs and 22 gauge for cats). Whole blood may be administered as such or mixed with normal saline to reduce viscosity. In both undiluted and diluted (mixed with normal saline) types, the blood should be mixed gently before administration and can be warmed gradually in a water bath up to 37° C to avoid hypothermia and cardiac arrhythmia in the host animal. Care must be taken not to heat blood too rapidly or too high to avoid RBC destruction.

Pharmacokinetics

Whole blood is usually administered in a large vein, but intraperitoneal or intramedullary (i.e. intraosseous) administration may also be used for neonate patients. After intraperitoneal administration, up to 40% of administered blood is absorbed in 24 hours and about 80% in 1 week. Intraosseous administration (mainly in femur or humerus) causes rapid absorption of blood (about 90% within 5 minutes). Storage and exogenous administration of blood lowers the life span of erythrocytes.

Side effects/Adverse effects

Adverse reactions to blood transfusions are serious and life-threatening. Immunological responses include both immediate (acute) and delayed (chronic) transfusion reactions. Immediate reactions include acute haemolysis due to reaction between donor and recipient antigens and antibodies. It occurs rarely with first transfusion, but is more likely to occur after repeated transfusions. Clinical signs of acute haemolysis include nausea, vomiting, salivation, tachycardia, hypovolaemia, urticaria and fever (an increase of 1°C over pretransfusion temperature). Delayed transfusion reactions usually occur within 7 to 10 days after transfusion and may include delayed haemolysis (shortened lifespan of RBCs) and jaundice. Delayed reactions also occur more with repeated transfusions due to sensitisation of the recipient to donor RBC antigens and also from WBC antigen-antibody reactions. Cross matching can help reduce the incidence of immunological transfusion reactions.

Non-immunological adverse effects of blood transfusion include sepsis from contaminated blood, hypocalcaemia from high citrate anticoagulant, hypervolaemia (circulatory overload) and air embolism. Urticaria, fever and vomiting are seen occasionally. Blood transfusion can also spread several diseases from donor to recipient such as babesiosis, anaplasmosis, feline or bovine leukaemia, and equine infections anaemia.

Contraindications and precautions

Collection, storage and transfusion of blood must be done aseptically. Cross matching should be done before all transfusions, essentially with repeated transfusions. Blood transfusion must always be done as per recommended route and dosage. Co-administration of other fluids should be avoided. Interspecies transfusions must not be recommended.

Clinical uses

Blood transfusion in animals is indicated in cases of acute haemorrhage or haemolysis, and also in the treatment of acute or chronic anaemia. Packed cell volume less than 20% in acute haemorrhage or anaemia and less than 10% in chronic anaemia often indicates requirement of whole blood transfusion. Animals with haemostatic disorders also require repeated transfusions of either whole blood or its components. In humans, blood transfusion is frequently required in patients suffering from hemophilia or sickle-cell disease.

Dose

a. The amount of whole blood required in different species depends on the PCV of donor and the PCV of recipient. It can be calculated from the given formula

$$\text{Amount of donor blood} = \text{Recipient weight} \times 80 \text{ ml/kg} \times \frac{\text{Desired PCV} - \text{Recipient PCV}}{\text{Donor PCV}}$$

Where, 80 ml/kg (70 ml/kg for cats) is the blood volume (~8%) in most domestic animals.

Example: Calculate the amount of donor whole blood (PCV 40%) required to obtain a PCV of 25% in a 20 kg

dog with existing PCV of 15%.

According to the formula

Amount of donor blood required (ml)

$$= 20 \times 80 \times \frac{25-15}{40} = 400 \text{ ml.}$$

Therefore, 400 ml of whole blood (with PCV of 40%) would be required to increase the recipient's PCV to the desired level.

b. In most species, the recommended dose of whole blood is generally 10 to 20 ml/kg, depending on the severity of the condition. At this dose, a 20 kg dog will require about 200-400 ml of whole blood.

Rate of administration

Regardless of the amount of blood to be transfused, the initial rate of transfusion should be slow i.e. 0.25 ml/kg during first 10 to 20 minutes. Then the animal should be carefully observed for any transfusion reaction. If found safe, the rate of blood transfusion for remaining period should be adjusted to 1 to 5 ml/kg/hour, although a higher rate (up to 20 ml/kg/hour) may also be used for hypovolaemic patients.

BLOOD COMPONENTS

Packed red blood cells

Packed red blood cells (Packed red cells, packed RBCs, packed cells, PRBCs) are a preparation of red blood cells which is transfused to correct low blood levels in anaemic patients. These are collected from whole blood that has either undergone centrifugation or been stored at 10°C until red blood cells have settled by sedimentation. This causes concentration of the red blood cells so that they occupy less space, thus the term "packed." As with the whole blood, packed RBCs should be either used within 24 hours or stored at refrigerated temperature (1° to 6° C) for subsequent usage. When stored properly, packed RBCs with a haematocrit between 70% and 80% will have the same expiration date as whole blood. Packed RBCs with a haematocrit greater than 80%, however, undergo accelerated ageing during storage and have a decreased mean survival time after transfusion. In special cases, PRBCs may be leuco-reduced, a process in which white blood cells are removed from the blood to reduce the possibility that patients will have immunological reactions to the transfusion.

Packed red blood cells are used in severe forms of anaemia, which generally do not require a simultaneous restoration of blood volume. It also reduces the danger of circulating overload and pulmonary oedema. Before administration, packed RBCs may be mixed with normal saline to reduce viscosity. Large-gauge needles should be used for administration of packed cells because forcing cells through small gauge needle may cause haemolysis of RBCs. Post-transfusion reactions are important adverse effects; therefore, cross matching is required before administration of packed RBCs. Similar to whole blood, dosage of packed RBCs is calculated from the donor PCV, recipient PCV and required PCV. One unit of packed RBCs typically will raise the haematocrit by 3-4% and the blood haemoglobin concentration by 1 gm/dl.

Plasma

Plasma that has been separated from cellular components such as RBCs is occasionally used in veterinary medicine for treatment of hypoalbuminaemia and also deficiency of clotting factors. Plasma contains many proteins, including blood clotting factors. Plasma harvested from fresh whole blood can be either used within 6 hours (called fresh plasma) or stored at -70° C (called fresh frozen plasma) for subsequent usage. Plasma may also be separated from previously collected blood up to its expiration date and stored frozen (called frozen plasma) for subsequent therapeutic use. Plasma (both fresh frozen and frozen) should be administered cautiously because it contains the majority of donor proteins including antibodies. As with whole blood, main adverse reactions are transfusion reactions.

Dose

Dogs & cats : 5 – 10 ml/kg, IV infusion at the rate of 2 to 5 ml/kg/hour.

Table 37.2: Storage of blood and blood components.

Component	Storage
1. Whole blood	
a. Fresh	< 12 hours (CPDA)
b. Stored	21 days (CPDA), 4° C
2. Packed red blood cells	21 days (CPDA), 4° C
3. Fresh frozen plasma	1 year, -30° C
	3 months, -18° C
4. Platelet rich plasma	1 – 3 days (CPDA), 22° C
5. Cryoprecipitate	1 year, -30°C
	3 months, -18°C

Abbreviation: CPDA = citrate phosphate dextrose adenine.

White blood cells

White blood cells are transfused to treat life-threatening infections in individuals those have a greatly reduced number of white blood cells or in patients in which white blood cells are functioning abnormally. The use of white blood cell transfusions is rare in both human and veterinary medicines because improved antibiotics and the use of cytokine growth factors have greatly reduced the need for such transfusions. White blood cells are obtained by hemapheresis (procedure in which blood is withdrawn, a component is separated and retained, and the remainder is retransfused into the donor) and can be stored for up to 24 hours.

Other blood components

Other blood components occasionally used in veterinary medicine include platelets and platelet rich plasma, antihaemophilic factors, fibrinogen and human serum albumin. Some of these blood components have been discussed earlier in Chapter 35.

BLOOD AND PLASMA SUBSTITUTES

Blood and plasma substitutes are agents which have characteristics similar to those of blood. They are used clinically as alternatives to blood or blood components.

1. Blood Substitutes

A blood substitute (also called artificial blood or blood surrogate) is a substance that mimics and functions like blood. It carries no blood component but has the ability to remove carbon dioxide and transfer oxygen to tissues. An ideal blood substitute should be non-toxic and non-immunogenic, free of

pathogens, capable of transporting oxygen and carbon dioxide, have a long shelf life, and be available at a reasonable cost. It should have characteristics similar to those of blood, including oxygen affinity, viscosity, plasma retention time and colloid osmotic pressure.

Oxygen-carrying blood substitutes, often called oxygen therapeutics, are commercially available in the form of haemoglobin based or perfluorocarbon based.

Oxyglobin

Oxyglobin is an ultra purified polymerised bovine haemoglobin in a modified lactate Ringer's solution. Oxyglobin is intended for the veterinary use and is mainly used to treat anaemia in dogs.

Chemistry and properties

Oxyglobin has an average molecular weight of 180 kDa. As such, it has colloidal properties similar to those of dextran 70 and hetastarch. The sterile oxyglobin solution contains 13 g/dL of haemoglobin, and has a pH of 7.8 and an osmolality of 300 mOsm/kg. The P50 value (partial pressure of oxygen when 50% of the haemoglobin is saturated) of oxyglobin is 38 mm Hg compared to 30 mm Hg of haemoglobin. Oxyglobin has low viscosity and a 2-year shelf life at ambient temperature of 2° C to 30° C. Because it is a polymerised haemoglobin, the molecules are much larger than the haemoglobin. It requires no cross matching or blood typing prior to use because it contains no red cell membrane.

Pharmacological effects

Oxyglobin as blood substitute delivers oxygen to tissues in a manner similar to that of red blood cells. The higher P50 value of oxyglobin than that of red blood cell haemoglobin allows the on-loading and off-loading of oxygen easier and thus facilitates delivery of oxygen to the tissue. Although the oxygen-carrying capacity of oxyglobin is not adequate for total blood replacement, its low viscosity makes it potentially useful in disorders characterised by abnormities in microcirculation. The improvement in arterial oxygen content following oxyglobin administration has duration of about 24 hours during which clinical signs associated with anaemia like lethargy/depression, exercise intolerance and tachycardia significantly improve. Oxyglobin mildly decreases PCV immediately after infusion and increases total and plasma haemoglobin concentration for about 24 hours.

Pharmacokinetics

Pharmacokinetic data on oxyglobin in domestic animals are limited. Similar to haemoglobin, oxyglobin is metabolised and eliminated via the reticuloendothelial system. Its intravascular half-life is dose-dependent, typically 30 to 40 hours at 30 ml/kg in healthy dogs. In therapeutic dosage, more than 90% of the administered drug is eliminated from body within 5 to 7 days after infusion. Less than 5% of oxyglobin existing as an unstabilised tetramer is cleared via the kidneys, resulting in transient haemoglobinuria.

Side effects/Adverse effects

Oxyglobin produces dose-dependent transient changes or side effects including yellow-orange discolouration of skin, mucous membranes, sclera and gums, red to dark-green discolouration of faeces, and brown-dark discolouration of urine. Gastrointestinal side effects including diarrhoea and vomiting may occur post-infusion. Circulatory overload may occur due to the plasma-extending properties of oxyglobin, especially if it is administered at an excessive rate or volume. Sometimes central venous pressure increases significantly within 2 to 7 hours of the onset of infusion and can develop into pulmonary oedema, if infusion is not decreased or interrupted. Antigenicity to bovine haemoglobin may result in formation of antibodies in heterogeneous species; anaphylactic reactions are possible.

Contraindications and precautions

Oxyglobin should not be used in patients those are known to be hypersensitive to it. It should be used with extreme caution in patients which have pre-existing cardiovascular disease or those are pre-disposed to pulmonary oedema. Treatment with oxyglobin results in volume expansion; therefore, PCV and RBC counts are not accurate measures of anaemia for 24 hours after administration. Once the container of oxyglobin is opened, the product should be used within 4 days, otherwise haemoglobin in product may get oxidised and its colour will change to brown. Administration of other colloidal solutions should be avoided concurrently with oxyglobin.

Clinical uses

Oxyglobin is indicated for the treatment of anaemia due to haemolysis, haemorrhage or ineffective erythropoiesis. Oxyglobin primarily acts as an oxygen-carrying bridge while the patient regenerates its red blood cells or until additional oxygen-carrying support in the form of whole blood is obtainable. Treatment success may be defined as the lack of need for additional oxygen carrying support (i.e. blood transfusion) for 24 hours after completion of infusion with oxyglobin. The patient should be monitored carefully for signs of circulatory overload by lung auscultation and by measuring central venous pressure and respiratory rate. The oxyglobin solution is administered by an intravenous set and catheter through a central or peripheral vein.

Dose

Dogs : 10 – 30 ml/kg, IV at a rate of up to 10 ml/kg/hour.

Hemopure

Hemopure is a chemically stabilized bovine haemoglobin preparation intended for human use. It is purified to remove or inactivate potential contaminants, including infectious agents (e.g. viruses and bacteria). It is similar to oxyglobin in action and is used in cases when blood is not available or is not an option. Hemopure may cause reactions and carries a risk of being used as a doping agent for performance enhancement in sports.

Other blood substitutes

Oxycyte, oxygent, perftoran and polyHeme are some oxygen carrying blood substitutes available mainly for human use. Oxycyte, oxygent and perftoran are perfluorocarbon (PFC) based emulsions designed to enhance oxygen delivery to damaged tissues. PolyHeme is a temporary oxygen-carrying blood substitute made from human haemoglobin that is currently in development for emergency treatment of trauma situations where large volumes of blood are lost.

2. Plasma Substitutes/Plasma Volume Expanders

Plasma substitutes or plasma volume expanders (plasma extenders, blood volume expanders, synthetic colloids, artificial colloids) are high molecular weight synthetic colloidal substances which attract and hold water in the vascular space, thereby

expanding vascular volume and exerting oncotic pressure in a manner similar to that of plasma proteins (Plasma substitutes or plasma volume expanders may also defined as synthetic colloids which restore circulating volume by mimicking the action of plasma proteins such as albumin). These substances, thus, do not enhance the oxygen- carrying capacity of blood, but merely replace the volume. Although natural colloids (e.g. plasma, albumin preparations and whole blood) are frequently used, synthetic colloids are more readily available and carry no risk of transmitting disease. The effectiveness of synthetic colloids is dependent on their physico-chemical properties such as molecular weight, colloid content and bioavailability, and their ability to expand intravascular volume and maintain oncotic pressure.

Desirable properties of a plasma volume expander

a. It should exert oncotic pressure comparable to that of plasma.
b. It should remain in circulation for required period and not readily leak out in tissues.
c. It should be pharmacologically inert.
d. It should not be pyrogenic or antigenic.
e. It should not adversely affect any visceral organ.
f. It should not interfere with grouping and cross matching of blood.
g. It should have longer storage period.
h. It should be easily sterilisable.
i. It should be readily available and economical.

Plasma volume expanders are useful in patients which are hypovolaemic and hypoproteinaemic, and in shock. They are often used in combination with hypertonic saline to increase the duration of effect of hypertonic saline and to reduce the volume of crystalloids needed to achieve and maintain adequate systemic arterial blood pressure and tissue perfusion. Plasma substitutes should not be used to maintain plasma volume in conditions such as burns or peritonitis where there is loss of plasma protein, water and electrolytes over periods of several days.

Dextrans/Dextran 70 and Dextran 40

Dextrans are complex polysaccharide molecules obtained from the action of *Leuconostoc mesentroides* bacterium on sucrose. Different molecular weight dextrans can be produced by acid hydrolysis of the parent macromolecule. Commercially, dextrans are available as dextran 70 (Mol. wt. 70,000), dextran 75 (Mol. wt. 75,000) and dextran 40 (Mol. Wt. 40,000). Dextran 70 is the most commonly used form in veterinary medicine followed by dextran 40. Dextran 70 is available as a white to light yellow amorphous powder and is freely soluble in water. Dextrans can be stored for about 10 years.

Chemistry and properties

Dextran 70 has an average molecular weight of 70,000 Da and is prepared as a 6% solution in 0.9% sodium chloride solution. It contains 60 g/L of colloid and 154 mEq/L each of sodium and chloride and has an osmolality of 310 mOsm/kg. Dextran 40 is prepared as a 10% solution in 0.9% sodium chloride and contains 100 g/L of colloid. Both dextrans 70 and 40 are hypertonic to plasma and, thus, expand plasma volume by drawing fluids into the vascular system. However, they do not promote the movement of water out of the cells.

Pharmacological effects

Dextran 70 has osmotic effects similar to those of albumin (Mol. wt. 69,000). Its colloidal osmotic effects draw fluids into the vascular system from the interstitial spaces, resulting in increased circulatory blood volume. It has been estimated that dextran 70 expands plasma volume by 1.38 times (138%) the volume infused. This occurs nearly for 24 hours. Dextran 40 produces a greater expansion of plasma volume (150% at 1 hour and about 200% at 2 hours) than the dextran 70, but the expansion exists for shorter duration (2 to 6 hours) because of its more rapid renal excretion.

Dextrans are not anticoagulants, but they have antithrombotic activity. They can interfere with coagulation of blood and platelet function and thus prolong bleeding time. Small molecules in dextran 40, in addition, reduce blood viscosity and prevent RBC slugging by coating the red blood cells. This improves microcirculation, reduces intravascular aggregation and may prevent thrombus formation. This property may be beneficial when treating patients at risk for thromboembolic disease. Blood glucose levels may get elevated during dextrans therapy due to their metabolism into glucose residues.

Pharmacokinetics

After IV infusion, dextrans increase circulating blood volume within one hour. This effect may persist for at least 24 hours with dextran 70 and for 2 to 6 hours with dextran 40. In dextran 70, approximately 20-30% of a given dose remains in the intravascular compartment at 24 hours and may be detected in blood even after 4-6 weeks after dosing.

Dextran 70 is slowly degraded to glucose by dextranase in the spleen, then metabolised to carbon dioxide and water. A small amount may be excreted directly into the gut and eliminated in the faeces. The dextran 40 is rapidly filtered at glomerulus because of its smaller molecular size. This is mainly responsible for its shorter duration and rapid removal from the intravascular space. Precipitation of dextran in the renal tubule due to absorption of tubular water may obstruct the tubules, causing acute renal failure.

Side effects/Adverse effects

Although dextrans used clinically are not antigenic, their structures are similar to other antigenic polysaccharides. Therefore, anaphylactic reactions may occur with both dextrans 70 and 40, but the incidence is relatively low. Dextrans may increase bleeding time and decrease von Willebrand's factor antigen and Factor VIII activity. Dextran 70 impairs coagulation more than dextran 40. Bleeding may occur more readily in patients with coagulation abnormalities such as thrombocytopenia. Dextran 40 has been associated with acute renal failure, which is more likely to occur when there is underlying renal disease or pre-existing dehydration. Dextran 70 has rarely been associated with acute renal failure. In humans, abdominal pain, nausea and vomiting have been associated with use of dextrans. Urticaria, itching, bronchospasm and fall in blood pressure occur occasionally.

Contraindications and precautions

Dextran use is contraindicated in patients with severe coagulopathies, severe anaemia and cardiac failure and in individuals hypersensitive to it. Dextrans should be carefully used in patients overly susceptible to circulatory load. Dextran 40 should not be used in patients suffering from pre-existing renal disease. Dextrans often interfere with blood grouping and cross

matching (because they can cross link with RBCs and appear as rouleaux formation), so these should not be done during dextran therapy. Patients on strict sodium restrictions should receive dextran cautiously as they are given in sodium solution, which contains appreciable amount of sodium.

Drug interactions

No clinically significant drug interactions have been reported with dextrans.

Clinical uses

Dextrans are most commonly used plasma volume expanders in both human and veterinary medicines. Dextran 70 is a relatively low cost synthetic colloid used for the adjunctive treatment of hypovolaemic shock. Dextran 40 is used also for the prevention and therapy of venous thrombosis. The use of dextrans and electrolyte solutions in preference to whole blood in the early stages of shock minimises the slugging phenomenon in peripheral microcirculation.

Dose

Dextran 70 and dextran 40

Dogs : 10 – 20 ml/kg/day, IV infusion at a rate not faster than 5 ml/kg/hour.

Cats : 5 – 10 ml/kg/day, IV infusion.

Hetastarch

Hetastarch (Hydroxyethyl starch, HES) is a synthetic complex mixture of ethoxylated amylopectin (starch) of various molecular sizes and is prepared by enzymatic cleavage of amylopectin molecules. Six percent hetastarch has a molecular weight range of 10,000 to 3,400,000 with an average weight 450,000. The colloidal properties of 6% HES solution in 0.9% sodium chloride solution approximate those of albumin. Its actions are similar to those of dextran 70 and provide an initial increase in plasma volume 1.37 times (137%) the volume infused. Hetastarch has also been shown to reverse changes in microvascular permeability and improve microcircular perfusion.

Metabolism and excretion of hetastarch depends on the size of various molecules present in it. Smaller molecules (Mol. wt. < 60,000) in hetastarch are excreted rapidly by kidneys as about 40% of infused dose appears in urine within 24 hours. Larger molecules are slowly hydrolysed by serum alpha-amylase into smaller molecules, which are then filtered through glomeruli over a period of 10-15 days. The degree of hydroxylation of the molecules delays the metabolism of hetastarch, thereby prolonging its excretion. About 20-30% of hetastarch molecules are extravasated and taken into cells of the reticuloendothelial system of liver, spleen and lymph nodes and metabolised slowly by proteolytic enzymes. A small amount of hetastarch is eliminated through the GI tract.

Potential concerns with hetastarch administration include allergic reactions. Rapid infusion of drug may induce nausea and vomiting. Hetastarch is also reported to induce coagulopathies, particularly in patients with pre-existing bleeding tendencies. Similar to dextrans, hetastarch has been used in animals as a plasma volume expander for the treatment of hypovolaemia during shock and as a systemic substitute in hypo-oncotic patients.

Dose

Dogs : 10 – 40 ml/kg/day, IV given in 5 – 10 mg/kg boluses until the end point of resuscitation (improvement in perfusion, increase in blood pressure, and normalisation of heart rate) is reached.

Cats : 5 – 10 ml/kg/day, IV; dose may be increased based on individual circumstances.

Oxypolygelatine/Gelatine

Oxypolygelatine is a 5.6% gelatine suspension in sodium chloride. It is produced from cattle bone marrow gelatine prepared by gradual controlled heating and oxidation with hydrogen peroxide. It has an average molecular weight of 30,000 Da and a molecular weight range of 5600 to 100,000 Da. Because of its lower mean molecular weight, oxypolygelatine differs from dextrans and hetastarch in several ways. It has two times the effect of the volume administered with the denser concentration of molecules per unit volume producing greater osmotic effect. Its elimination is by direct renal filtration, although extrarenal mechanisms including gastrointestinal excretion and metabolism also occur. It has shorter plasma half-life of 2 to 4 hours. Oxypolygelatine has no direct effect on coagulation proteins or platelets and is also not associated with renal failure.

Anaphylactic reactions are potential side effects of oxypolygelatine. Dilutional coagulopathies may occur following large volume administration. Oxypolygelatine has been reported to significantly lower the serum calcium concentrations, initiating the tetany. For this reason, the oxypolygelatine products for veterinary use are often supplemented with calcium. Like other synthetic colloids, it should be used with extreme care in animals susceptible to circulatory overloading.

Dose

Dogs : 10 – 20 ml/kg, IV.

Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a synthetic complex organic polymer with molecular weight ranging from 30,000 to 50,000 Da (average molecular weight 40,000 Da). It is less commonly used as plasma volume expander because it causes release of histamine and also is stored in small fraction in the cells of reticuloendothelial system for a very long time.

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SECTION-VI

DRUGS AFFECTING RENAL FUNCTION AND FLUID-ELECTROLYTE BALANCE

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CHAPTER 38 GENERAL CONSIDERATIONS

- Renal functions
  - Physiology of kidney
    - Glomerular filtration
    - Tubular transport
    - Tubular mechanism of renal epithelial transport
    - Sites of tubular transport
      - Proximal convoluted tubule loop of Henle
        - Descending loop of Henle
        - Ascending limb of the loop of Henle
      - Distal convoluted tubule
      - Collecting tubule and collecting duct
    - Hormonal and chemical transmitter control of renal function
- Fluid, electrolyte and acid-base balance
  - Solution
    - Percentage or Percent solution
    - Normality or Normal solution
    - Molarity/Molar solution
    - Molality/Molal solution
    - Equivalent
  - Osmosis and osmotic pressure
    - Osmosis
    - Osmotic pressure
    - Tonicity
    - Types of solutions
    - Hydrogen ion concentration and pH
      - Buffer solution

RENAL FUNCTIONS

The renal system is made up of two kidneys, two ureters, urinary bladder and urethra. The main function of the kidney is excretion of waste products such as urea, uric acid and creatinine. It also plays an important role in the acid-base balance and regulation of salt and electrolyte content and volume of extracellular fluid. In addition, production and release of various hormones, autacoids and enzymes by the kidney play a vital role in the control of systemic blood pressure and red blood cell production.

Physiology of Kidney

The basic urine-forming unit of kidney is the nephron. Each nephron is composed of an initial filtering component, the renal corpuscle, and a specialized long tubule, the renal tubule. The renal corpuscle is composed of glomerulus, a collection of capillaries, and Bowman's capsule, a capsule that surrounds the glomerulus. The renal tubular portion of nephron broadly consists of proximal tubule, loop of Henle (both ascending and descending limbs) and distal tubule. The basic function of renal corpuscle is to filter out large solutes from the blood and then deliver water and small solutes to the renal tubule for modification. In the cortex, nephrons join with collecting duct system, where final alterations of the tubular fluid take place in the formation of urine.

Glomerular filtration

The glomerular filtration is a process by which water and small solutes are passed from the lumen of glomerular capillary to the space of Bowman's capsule. In this process, all the plasma constituents gain access to the glomerular capsule with exception of proteins, lipids and substances bound to proteins. The force for glomerular filtration is provided by the hydrostatic pressure (60% of the arterial pressure) of blood derived from the work of heart. However, greater than 99% of the glomerular filtrate is reabsorbed from the tubular portion of nephron. This mainly occurs to reabsorb certain essential constituents like water, electrolytes and nutrients present in the glomerular filtrate, which have to be conserved. The glomerular filtration rate (GFR) in a normal animal is maintained within the physiological range by renal modulation of systemic blood pressure and intravascular volume, and by intrinsic control of renal blood flow and glomerular capillary pressure. The renin-angiotensin-aldosterone system is an important mechanism of control of GFR and renal blood flow.

Tubular transport

The tubular cells perform the active secretory and resorptive functions and also allow the passive diffusion of solute in direction appropriate to its concentration or electrochemical gradient. From the pharmacological point of view, the most important tubular mechanisms of electrolyte transport are reabsorption of sodium and chloride, and secretion of H^+ and K^+. In addition, there are related but separate mechanisms for the transport of Ca^{++}, Mg^{++}, phosphate, sulphate, and organic acids and bases. Overall the mechanisms involved in ion movement across tubular cells are complex and involve a variety of transport mechanisms. Further, the rate of absorption and secretion of filtered substances varies among segments of the renal tubule.

Tubular Mechanism of Renal Epithelial Transport

Tubular transport of solute occurs mainly by six different processes. The kinds of transport achieved in a particular nephron segment depend mainly on the types of transporter present and whether they are present in the luminal or epical membrane. Some

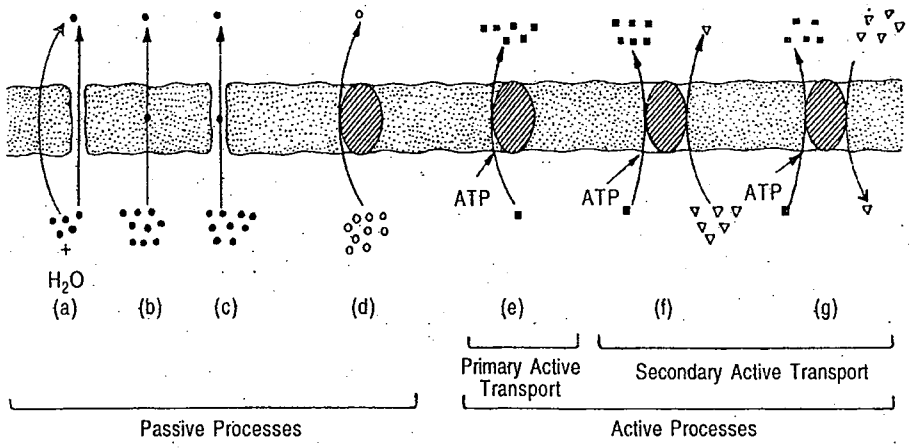


Fig. 38.1. Schematic representation of tubular transport of solutes (see text for detail).
(a) Solvent drag (b) Simple diffusion (c) Pore filtration (d) Facilitated transport (e) Primary active transport (f) Symport active transport (g) Antiport active transport.

of these transport mechanisms have been discussed earlier for drugs in Chapter 4.

*a. **Simple diffusion:*** Simple diffusion of solutes with sufficient lipid solubility across the renal epithelial cell membrane occurs passively down their electrochemical gradient. The mechanism is similar to that for drugs discussed in Chapter 4.

*b. **Channel mediated diffusion*:** Many solutes, particularly ions (e.g. Na^+, K^+ and Cl^-) diffuse passively across the renal epithelial membrane through ion-channels or pores. These channels are formed by proteins embedded in the cell membrane and are similar in composition and characters to ion channels present in other organs.

*c. **Solvent drag/Convection solute flow:*** It is the convection flow in which dissolved solutes are simply dragged/carried across a membrane (either through aqueous pores or between adjacent cells) by bulk flow. It is a very simple process and is similar to pore filtration discussed earlier for drugs.

*d. **Facilitated transport/Uniport*:** It is a carrier-mediated process in which solute is transported down electrochemical gradient. In this process, the solute binds to the integral protein (carrier) and is transferred across the cell membrane without utilisation of energy (Figure 38.1).

*e. **Active transport/Primary active transport:*** It is a carrier mediated process in which solute is transported against electrochemical gradient with ATP hydrolysis providing the driving force.

*f. **Secondary active transport:*** It is a carrier-mediated transport in which one solute is moved against the electrochemical gradient by utilizing energy and another solute(s) is moved against/down the electrochemical gradient by utilizing free energy of first solute. It is of two types:

(*i*) *Symport transport/Co-transport:* It is the transport of two solutes in the same direction by utilizing the same carrier. In this type, energy is utilized for the movement of one solute against its electrochemical gradient, and the other solute is moved against/down the electrochemical gradient simply by occupying the carrier (e.g. Na^+-glucose, Na^+-P_i and Na^+-amino acid co-transport).

(*ii*) *Antiport/Counter-transport:* It the secondary transport in which two substances move in the opposite directions. In this process, ATP is utilised to create an electrochemical gradient for a given solute and the free energy of that solute in then released to transport other solute in opposite direction (e.g. Na^+-H^+ counter transport).

SITES OF TUBULAR TRANSPORT

In general, tubular transport of substances occurs from four sites - proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting tubule and duct.

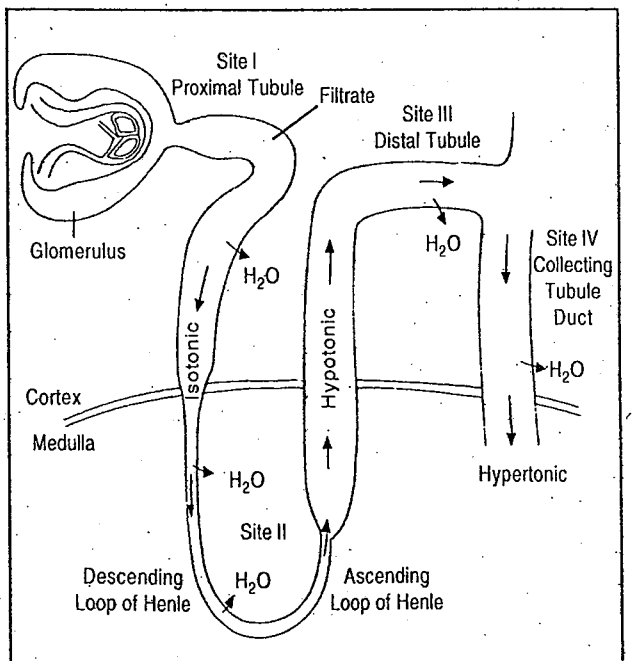


Fig. 38.2. Schematic representation of the nephron showing four sites of solute absorption.

Site I: Proximal Convoluted Tubule

The proximal tubule is responsible for reabsorption of the ultra filtrate to a greater extent than remainder part of the tubule. It absorbs almost all of glucose, HCO_3^-, amino acids and other metabolites. Approximately two thirds of the Na^+ is reabsorbed in the proximal tubule and chloride and water follow it passively to maintain electrical and osmolar equality. In general, transport through the transcellular pathway largely occurs by carrier-mediated transport, while the paracellular transport occurs by passive diffusion or by solvent drag.

In proximal tubule, much of the transport of substances from the tubular fluid to the blood is driven by active transport of Na^+ by a Na^+, K^+-ATPase (Na^+-K^+-pump) located in the basolateral plasma membrane. It is an antiport (counter-transport) mechanism that moves 3 Na^+ ions out of cell into the interstitial fluid against a concentration gradient and allows 2 K^+ into the cell also against concentration gradient for each ATP hydrolysed. Due to the existence of large intracellular to extracellular gradient of K^+, the K^+ diffuses out through K^+ channels (Figure 38.3) making the interior of cell electrically negative to the exterior. This establishes an electrochemical gradient for Na^+ across the apical membrane, encouraging movement of Na^+ from the tubular fluid into the cell.

Sodium

The movement of Na^+ across the apical membrane is facilitated by a variety of specific transporters located in the membrane, which carry other solutes in the same direction as Na^+ (symport or co-transport) or in the opposite direction (antiport or counter transport). Broadly, four mechanisms of Na^+ transport have been defined in the proximal convoluted tubule.

a. Sodium may diffuse across the luminal membrane via Na^+ channels into the epithelial cell down the electrochemical gradient for Na^+ that is established by the basolateral Na^+-K^+-ATPase.

b. Transport of Na^+ coupled to active reabsorption of glucose, amino acids, other organic anions and PO_4^{-3} against their electrochemical gradient by specific symporters (e.g. Na^+-glucose, Na^+-amino acid and Na^+-P_i). This active process results in movement of Na^+ and co-transported solutes out of the tubular lumen into the cell. Further, increased intracellular concentrations of these co-transported substances allow them to move across the basolateral membrane and into blood by passive or facilitated diffusion.

c. The Na^+ is reabsorbed in exchange with an intracellular proton (H^+). The Na^+ and H^+ counter transport across apical plasma membrane occurs via a Na^+-H^+ antiporter located in the luminal membrane and is driven by the chemical gradient for Na^+. The secreted H^+ combines in the tubular fluid with filtered HCO_3^- to form carbonic acid (H_2CO_3), which dissociates to form H_2O and CO_2 with the help of enzyme carbonic anhydrase present in the apical membrane. The CO_2 and H_2O diffuse passively into the cell, where they combine with H_2O to again form H_2CO_3. This intracellular hydration of CO_2 is catalysed by cytoplasmic carbonic anhydrase. The H_2CO_3 dissociates to form H^+ and HCO^- within the cell. The H^+ is transported into the tubular fluid through Na^+-H^+ antiporter, while HCO_3^- moves across the basolateral membrane and back into the blood through a Na^+-$3HCO_3^-$ co-transporter and possibly a HCO_3^--Cl^- antiporter.

d. As above-mentioned processes selectively reabsorb the Na^+-HCO_3^-, glucose, amino acids and other solutes and water follows them, the concentration of Cl^- in tubular fluid rises. This creates passive driving force for Cl^- to diffuse through the paracellular pathway, particularly in the later proximal tubule. As Cl^- moves passively down its concentration gradient, it carries Na^+ along with it due to electrostatic attraction.

Other solutes and water

Reabsorption of Cl^- generally follows reabsorption of Na^+ in the renal tubule. In the early proximal tubule, both chemical and electrical gradients favour the reabsorption process, mainly by passive paracellular transfer of Cl^- through the highly permeable zonula occludens (a highly permeable structure/epithelium that attaches proximal tubule cells to each other). Some Cl^- also crosses the luminal membrane via antiport mechanisms. It is believed that majority of K^+ (~80-90%) and Ca^{++} (~70%) are reabsorbed in the proximal tubule by passive mechanisms including solvent drag and passive diffusion down the electrical gradient. A small amount of Ca^{++} (also K^+) reabsorption also occurs by an active, transcellular pathway in the proximal tubule. Inorganic phosphate (P_i) is also largely reabsorbed (80%) by the proximal tubule. A large proportion of filtered peptides and low

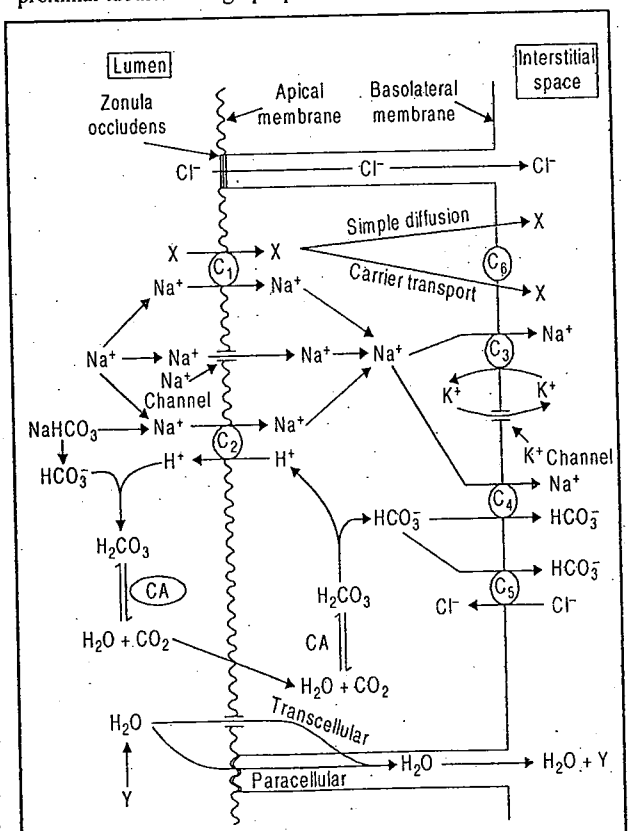


Fig. 38.3. Schematic representation of tubular transport of substances in the proximal tubule.

Abbreviations : C_1 = Na^+ – X – symporter ; C_2 = Na^+ – H^+ antiporter; C_3 = Na^+- K^+ - ATPase (sodium pump) ; C_4 = Na^+ – HCO_3^- symporter ; C_5 = HCO_3^- – Cl^- antiporter; C_6 = transporter for X ; X = amino acid, glucose or inorganic phosphate ; Y = membrane permeable solute in water for solvent drag absorption ; CA = carbonic anhydrase enzyme.

molecular-weight proteins are first degraded to amino acids by peptidases present in the proximal tubule brush border and then reabsorbed by co-transport with Na^+, as discussed previously.

In proximal tubule, the epithelium is highly permeable to water. Therefore as the solute (Na^+ and Cl^-) is reabsorbed, water diffuses along the osmotic gradient with such rapidity that the tubular fluid and the adjacent peritubular or interstitial fluid maintains approximately the same osmolality. Water moves both through aqueous pores in the luminal and basolateral cell membranes as well as through the tight junctions (paracellular pathway).

Organic acid and base secretion

The proximal tubule has a highly efficient transport system for organic acids and an equally efficient but separate transport system for organic bases. Both systems are powered by a sodium pump (Na^+-K^+-ATPase) in the basolateral membrane. This group of organic substances include both endogenous waste products (e.g. bile acids, urate and oxalate) and exogenous drugs and toxicants (e.g. penicillin G, diuretics and paraquat). In birds, tubular secretion plays a larger role than in mammals because majority of uric acid, the end product of protein metabolism, is excreted by tubular secretion.

Site II: Loop of Henle

The loop of Henle plays an important part in regulating the osmolality of urine and hence in regulating the osmotic balance of body. The loop of Henle consists of a descending and an ascending portion.

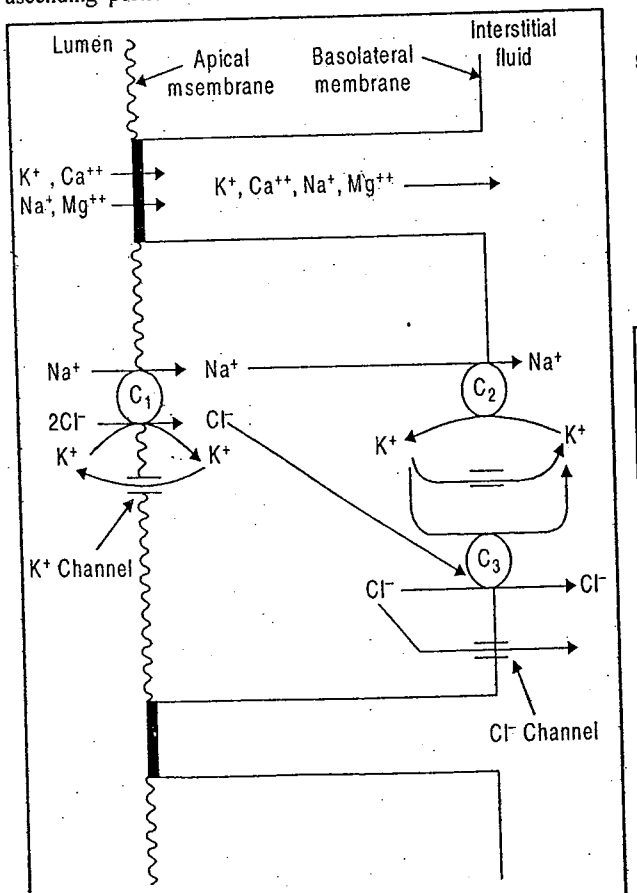


Fig. 38.4. Schematic representation of tubular transport of substances in the ascending limb of loop of Henle.
Abbreviations : C_1 = Na^+-K^+-$2Cl^-$ symporter ; C_2 = Na^+-K^+ – ATPase (sodium pump); C_3 = Na^+ – Cl^- symporter.

a. **Descending loop of Henle:** The thin descending loop of Henle is highly permeable to water, which is passively absorbed from this part under the influence of osmotic forces. These forces arise because the interstitial fluid of the medulla is hypertonic. The tall epithelium of proximal tubule with extensive brush border and abundant mitochondria abruptly changes to low smooth and simple epithelium with few mitochondria. So active transport of solutes in this segment is virtually non-existent.

b. **Ascending limb of the loop of Henle:** The thick ascending limb of loop of Henle is almost impermeable to water but it absorbs salt actively and thus dilutes the tubular fluid. In the medullary portion of ascending limb, Na^+ and Cl^- are actively absorbed (~25-30%) by a Na^+-K^+-$2Cl^-$ co-transport system (Figure 38.4) without accompanying water. This process is driven by the electrochemical gradient for Na^+ produced by the Na^+-K^+-ATPase in basolateral membrane. Intracellular chloride then passes out of the cell by diffusion via Cl^- channels into the interstitial fluid. In addition, a K^+-Cl^- symporter in the basolateral membrane permits co-transport of Cl^- down an electrochemical gradient with concomitant transport of Na^+ against an electrochemical gradient. Most of the K^+ taken into cell by the Na^+-K^+-$2Cl^-$ symporter cycles back into lumen through K^+ channels across the apical membrane, but some K^+ is reabsorbed along with Mg^{++} and Ca^{++} mainly via a paracellular pathway.

Site III: Distal Convoluted Tubule

The cells of distal convoluted tubule and also cortical portion of ascending limb of loop of Henle are impermeable to water but continues to absorb Na^+ and Cl^- through a separate Na^+-Cl^- symporter resulting in further dilution of tubular fluid. The Na^+-Cl^- symporter permits active transport of Na^+ across the apical membrane from tubular fluid down the chemical gradient for Na^+ generated by basolateral Na^+-K^+-ATPase. Chloride follows the

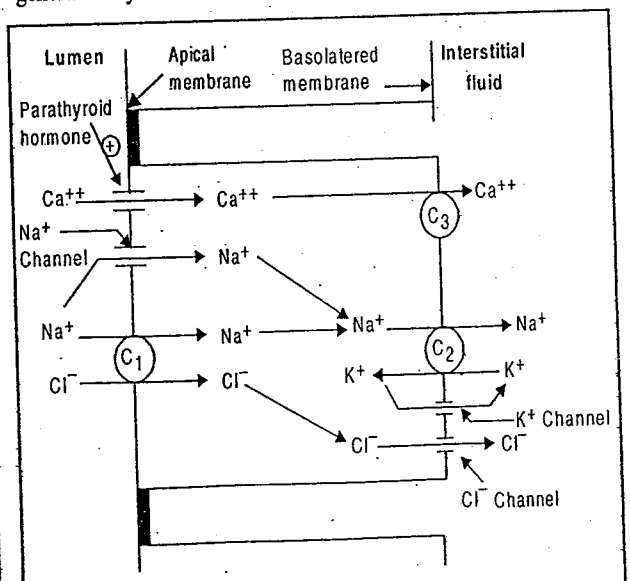


Fig. 38.5. Schematic representation of tubular transport of substances in the distal tubule.
Abbreviations : C_1 = Na^+ – Cl^- symporter ; C_2 = Na^+-K^+ – ATPase ; C_3 = Ca^{++} – ATPase ; + = activation.

sodium passively. The Cl^- that is transported into cell moves to interstitial fluid via a basolateral Cl^- channel, driven by the electrochemical gradient. Some Na^+ is also absorbed from lumen via Na^+ channels in the apical membrane (Figure 38.5). In addition to Na^+ and Cl^-, the distal convoluted tubule and connecting tubule absorb the remaining Ca^{++} via a transcellular pathway, which is modulated by parathyroid hormone (PTH). Parathyroid hormone appears to increase Ca^{++} channels in the luminal membrane, thereby causing passive reabsorption of Ca^{++}. Intracellular Ca^{++} is extruded across the basolateral membrane by a Ca^{++}-ATPase and via Na^+-Ca^{++} antiport. The reabsorption of salts without concurrent water absorption results in a hypotonic tubule fluid (Figure 38.2) and, thus, both the distal convoluted tubule and the thick ascending limb are sometimes called the diluting segments. The dilution of tubule fluid takes place irrespective of the volume status of the animal.

Site IV: Collecting Tubule and Collecting Duct

The collecting duct system begins with the connecting segment, which is a transfusion region from distal convoluted tubule to initial collecting duct. Throughout most of collecting duct system, there exist two main cell types - the principal cell and the intercalated cell. The principal cell is primarily responsible for Na^+ and Cl^- reabsorption in the collecting duct as it has extensive basolateral membrane infolding that contains Na^+-K^+-ATPase. The Na^+ channels in the luminal membranes of principal cells provide a conductive pathway for entry of Na^+ into the cell down electrochemical gradient created by the basolateral Na^+-K^+-ATPase. This results in a lumen negative electrical potential that can drive Cl^- absorption passively through the paracellular pathway and/or transcellularly (species specific). At the same time, K^+ is pumped actively from the interstitial fluid into the cell by Na^+-K^+-ATPase, raising the intracellular K^+ concentration above that of the interstitial fluid and the tubule fluid. Unlike other tubular segments already discussed (proximal tubule and ascending limb of loop of Henle), more of intracellular K^+ is secreted into the lumen via K^+ channels in the luminal membrane (Figure 38.6). This occurs because the apical K^+ channel is more permeable than the basolateral K^+ channel and the lumen negative potential provides a driving force for K^+ secretion into the lumen.

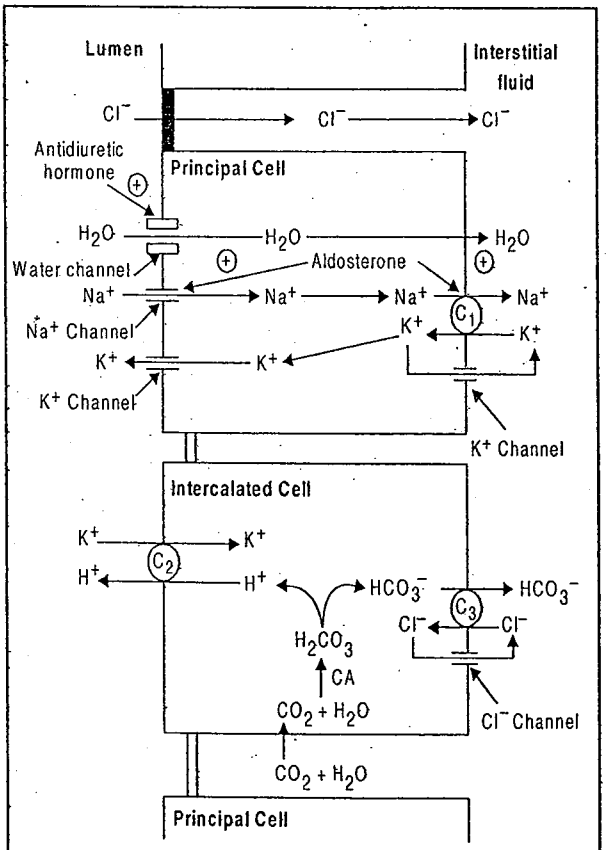


Fig. 38.6. Schematic representation of tubular transport of substances in the collecting tubule and collecting duct.
Abbreviations : C_1 = Na^+-K^+ – ATPase ; C_2 = H^+-K^+ – ATPase (proton pump); C_3 = HCO_3^- – Cl^- antiporter ; CA = carbonic anhydrase.

In the connecting segment cells and principal cells of collecting duct, the Na^+ absorption and also K^+ excretion, driven by Na^+-K^+-ATPase, is mainly under the influence of aldosterone. Aldosterone secretion from adrenal cortex is stimulated by systemic hypotension (also hyperkalaemia) through the renin-angiotensin system to enhance Na^+ reabsorption, which in turn enhances water reabsorption in order to correct volume depletion. At cellular level, aldosterone increases the permeability of the apical membrane Na^+ channels and stimulates Na^+-K^+-ATPase, thereby enhancing Na^+ reabsorption. Chronic administration of aldosterone may also increase the number of basolateral sodium pumps and also K^+ channels in the apical membrane (see also Diuretics, Chapter 39).

Unlike principal cells, the intercalated cells in collecting duct are responsible for K^+ reabsorption. Potassium ions are actively transported across the apical membrane in exchange for hydrogen ions by a H^+-K^+-ATPase (proton pump) similar to that in the parietal cell of stomach.

Other substances and water

Urea is reabsorbed from the medullary section of the collecting tubule and passed into the interstitial fluid. Bicarbonate is exchanged for H^+ as in the proximal tubule. In this portion of nephron, the zona occludens has low permeability to both ions and water, individual regulation of which can be influenced by hormones. The absorption of water is under the influence of antidiuretic hormone (ADH, vasopressin). The ADH produces a sustained increase in permeability of water in this part of nephron, allowing its passive diffusion (Figure 38.6). The antidiuretic hormone is secreted by the posterior pituitary and binds to its receptors (V_2) in the basolateral membrane causing an increase in number of water channels in the apical membrane (see for detail Chapter 49). In the presence of ADH, the filtrate (urine) equilibrates with the hyperosmotic interstitial fluid and is then discharged at the end of collecting duct as a hypertonic or concentrated solution. In absence of ADH, the nephron becomes impermeable to water and urine remains hypoosmotic.

Hormonal and Chemical Transmitter Control of Renal Function

Several chemical transmitters and hormones influence the tubular transport of solutes and water in a nephron. These include norepinephrine, prostaglandins, natriuretic hormone, antidiuretic hormone, parathyroid hormone, angiotensin II and aldosterone. Effects of these mediators on renal functions have been summarised in Table 38.1.

Table 38.1: Chemical transmitters and hormones regulating renal function.

Agent	Source/Distribution	Site of Action	Function
Norepinephrine	Renal arterioles	All parts of nephron.	Renal vasoconstriction, increased renin release, decreased GFR and increased Na^+ absorption.
Angiotensin II	Kidneys	Distal convoluted tubule.	Increased Na^+ and water retention, enhanced aldosterone release.
Aldosterone	Adrenal cortex	Distal convoluted tubule.	Enhanced reabsorption of Na^+ and water, and excretion of H^+ and K^+.
Prostaglandins	Kidneys	All parts of nephron.	Modulates renal circulation and renin release. Prostacyclin increases excretion of Na^+ and K^+; PGE_2 inhibits action of ADH and increases excretion of Na^+, K^+ and Cl^-.
Anti-diuretic hormone	Supraoptic nucleus of hypothalamus	Collecting tubule and duct.	Enhanced reabsorption of water.
Atrial natriuretic peptide	Atria of heart	Proximal tubule	Reduced reabsorption of Na^+ (natriuresis) and also water.
Parathyroid hormone	Parathyroid gland	Distal convoluted tubule	Increased reabsorption of Ca^{++} and excretion of PO_4^{-3}.

Abbreviations: ADH = anti-diuretic hormone; GFR = glomerular filtration rate; PGE_2 = prostaglandin E_2.

FLUID, ELECTROLYTE AND ACID-BASE BALANCE

The maintenance of a relatively constant volume and a stable composition of body fluids are essential for homeostasis. This is precisely regulated by a balance between the input and the output of water and electrolytes. Similarly, precise control of hydrogen ion is essential because activities of almost all enzyme systems in body are influenced by hydrogen ion concentration. Disturbance of water, electrolyte and acid-base balance are associated with many diseases. The kidney is the major regulator of water, electrolytes and acid-base balance.

I. SOLUTION

A solution is a physically homogenous liquid mixture of two or more substances. In such a mixture, a solute is dissolved in another substance known as solvent. The concentration of a solute in solution is usually expressed as percentage, normality, molarity, molality and equivalent.

1. Percentage or Percent solution (%)

Percent solutions (%) are equal to parts per hundred or the amount of solute per 100 total units of solution. A 1% solution is prepared by dissolving 1 g of a solid or 1 ml of a liquid in sufficient amount of solvent to give 100 ml of the solution.

For example :

1% NaCl = 1 g NaCl/ 100 ml of solution, or 1 g NaCl/100 ml.

2. Normality or Normal solution (N)

Normality or Normal solution (N) is the number of gram equivalent weight per litre of solution. A 1 N solution is prepared by dissolving 1 g equivalent weight of a solute in sufficient amount of solvent to give 1 litre of the solution.

$$\text{Where equivalent weight (Eq. wt)} = \frac{\text{Molecular weight}}{\text{Valence}}$$

For example :

(*i*) NaCl (Mol. wt = 58.5, valence =1)

$$\text{Equivalent weight} = \frac{58.5}{1} = 58.5$$

1 N NaCl = 58.5 g NaCl/L

(*ii*) H_2SO_4 (Mol. wt. = 98; valence = 2)

$$\text{Equivalent weight} = \frac{98}{2} = 49$$

1 N H_2SO_4 = 49 g H_2SO_4/L

3. Molarity/Molar solution (M)

Molarity or Molar solution (in units of mol/L, molar, or M) is the number of moles per litre of solution. A 1 molar solution is prepared by dissolving 1 mole (mol) of a solute in sufficient quantity of solvent to give 1 litre of solution.

Where 1 mole (mol) = 1 gram molecular weight or weight of a substance in grams equal numerically to its molecular weight.

A dilute solution is usually expressed as millimole (mMol), which is one thousandth of the mole i.e. milligram molecular weight.

For example :

(*i*) NaCl (Mol. wt. = 58.5)

1 Mol = 58.5 g

1 mMol = 58.5 mg

1 Molar (M) NaCl = 58.5 g NaCl /L

(*ii*) H_2SO_4 (Mol. wt. = 98)

1 Mol = 98 g

1 mMol = 98 mg

1 Molar (M) H_2SO_4 = 98 g H_2SO_4/L

4. Molality/Molal solution

Molality or molal (mol/kg, molal, or *m)* solution is the number of moles of a solute per kilogram of solvent. A 1 molal solution is prepared by dissolving 1 mole of a solute in 1 kilogram of solvent.

For example :

NaCl (Mol. wt = 58.5)

1 Molal NaCl = 58.g NaCl/kg

5. Equivalent

One equivalent (Eq) of a substance is the atomic weight divided by ionic valence. As most electrolytes in biological fluids are present in dilute concentrations, it is more convenient to express their concentrations as milliequivalent which is one-thousandth of equivalent.

Equivalent or milliequivalent is generally used to express concentration or strength of electrolytes in body because electrolytes in solution combine in proportion to their ionic valance (equivalent to equivalent) rather than to their weights (gram to gram, or mole to mole). This is because the maintenance of electrical neutrality in biological fluids requires that there be an equal number of equivalents of anions and cations in solution. Accordingly, multivalent ions have greater chemical combining power than the univalent ions

e.g. one divalent ion will react with two univalent ions.

$$1 \text{ Equivalent (Eq)} = \frac{1 \text{ gram atomic weight}}{\text{Valence}}$$

$$1 \text{ Milliequivalent (mEq)} = \frac{1 \text{ milligram atomic weight}}{\text{Valence}}$$

For example :

a. Univalent ions

For all univalent ions, 1 mole = 1 Equivalent (1 Mol = 1 Eq), and 1 millimole = 1 milliequivalent (1mMol = 1 mEq).

(*i*) Sodium (Na^+; atomic weight = 23)

$$1 \text{ Equivalent (Eq)} = 23 \text{ g } \frac{23 \text{ g}}{1} = 23 \text{ g}$$

$$1 \text{ Milliequivalent (mEq)} = \frac{23 \text{ mg}}{1} = 23 \text{ mg}$$

(*ii*) Potassium (K^+; atomic weight = 39)

$$1 \text{ Equivalent (Eq)} = \frac{39 \text{ g}}{1} = 39 \text{ g}$$

$$\text{Milliequivalent (mEq)} = \frac{39 \text{ mg}}{1} = 39 \text{ mg}$$

b. Divalent ions

For all divalent ions, 1 mole = 2 Equivalents (1 Mol = 2 Eq) and 1 millimole = 2 milliequivalents (1 mMol = 2 mEq).

(*i*) Calcium (Ca^{++}; atomic weight = 40)

$$1 \text{ Equivalent (Eq)} = \frac{40 \text{ g}}{2} = 20 \text{ g}$$

$$1 \text{ Milliequivalent (mEq)} = \frac{40 \text{ mg}}{2} = 20 \text{ mg}$$

(*ii*) Magnesium (Mg^{++}; atomic weight = 24)

$$1 \text{ Equivalent (Eq)} = \frac{24 \text{ g}}{2} = 12 \text{ g}$$

$$1 \text{ Milliequivalent (mEq)} = \frac{24 \text{ mg}}{2} = 12 \text{ mg}$$

As laboratory results are usually expressed in terms of mg/100 ml (mg%), the conversion to mEq/L can be made from the following formula:

$$\text{mEq/L} = \frac{\text{mg} / 100 \text{ ml}}{\text{Atomic wt.}} \times \text{Valence}$$

However, strength of electrolytes in body fluids in milliequivalent (mEq/L) is not ideally applicable to all electrolytes in all circumstances. For example, serum phosphorus consists of variable proportions of phosphate and monohydrogen and dihydrogen phosphate, so that no valence can be assigned to it. Similarly, ionised (free or diffusible) calcium fraction is given by calcium not bound to plasma proteins. Since usual laboratory estimations include all the serum calcium, including protein bound ions, it is not proper to express the ions as mEq/L. Plasma proteins are generally expressed as g/dL although they are often included in anions.

II. OSMOSIS AND OSMOTIC PRESSURE

All fluid compartments are separated by semipermeable membranes, which allow free passage of water in either direction, but restrict particle, ion, crystalloid and colloid content. Whenever there is a higher concentration of solute on the one side of cell membrane, water diffuses across the membrane toward the region of higher solute concentration so that concentration of solutes on both sides of the membrane remains equal. Because the concentration of a solution depends on the number of solute particle in solution, the number of individual particles in solution is taken into account irrespective of their electrical charge, valence or chemical structure.

Osmosis

Osmosis is the net diffusion of water from a region of high water concentration (low solute concentration) to one that has a lower water concentration (high solute concentration). It may also be used to describe a physical process in which any solvent moves, without input of energy, across a semipermeable membrane (permeable to the solvent, but not the solute) separating two solutions of different concentrations. Osmosis is important in biological systems, as many biological membranes are semipermeable. In general, these membranes are impermeable to organic solutes with large molecules such as polysaccharides, while permeable to water and small, uncharged solutes.

Osmotic pressure

Osmotic pressure is the precise amount of pressure required to prevent osmosis; or it is the amount of pressure that must be applied to prevent the net diffusion of water through a membrane. Animal cells have an osmotic pressure approximately equal to that of circulating blood; therefore, fluids normally do not flow out of or into the intracellular compartment (see also isotonic solution). The osmotic pressure of a solute in solution is related to the number of particles of that solute in solution, and not its weight or its charge. For example, one molecule of albumin (molecular wt. 69,000) exerts the same osmotic pressure as one molecule of glucose (molecular wt. 180) or sodium (molecular wt. 23), and divalent and trivalent ions exert same osmotic pressure as univalent ions. However, substances those give two or more particles (e.g. $NaCl \rightarrow Na^+ + Cl^-$), create higher osmotic pressure.

Osmotic pressure exerted by small non-permeable ions/solutes (crystalloids) is simply called the osmotic pressure, while the osmotic pressure that results from presence of large, non-permeable molecules (colloids) at the membrane is called either colloid osmotic pressure or oncotic pressure. Sodium, the highest concentrated ion in ECF, is the most important crystalloid contributing to the osmolality, while proteins (particularly albumin) are the most osmotically active colloid. Osmotic pressure is expressed in terms of osmolarity or osmolality.

Osmolarity and osmolality: Osmolarity is the measure of solute concentration, defined as the number of osmoles (Osm, osmol) of solute per litre (L) of solution (osmol/L or Osm/L). In a similar way, osmolality may be defined as a measure of the number of osmoles of solute per kilogram of solvent (osmol/kg or Osm/kg).

Where, osmole refers to the number of osmotically active particles in solution. Osmotically active solutes/particles in the body fluids include sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphates, sulphates, lactates, creatinine, phosphocreatinine, amino acids, adenosine triphosphate, glucose, proteins, urea, etc.

$$1 \text{ Osmole (Osm)} = \frac{1 \text{ Gram molecular weight}}{\text{Number of particles per molecule}}$$

As most solutes in body fluids are present in relatively dilute concentrations, it is more convenient to express concentrations as milliosmoles (mOsm), which is one thousandth of the Osm.

$$1 \text{ Milliosmole (mOsm)} = \frac{1 \text{ milligram molecular weight}}{\text{Number of particles per molecule}}$$

Osmolarity (or Osmolality) is distinct from molarity (or molality) because it measures moles of solute particles rather than moles of solute. The distinction arises because some compounds can dissociate in solution, whereas others cannot. The compounds which dissociate (e.g. ionic compounds) give two or more particles in solution, all of which contribute to the osmotic pressure of the solution. The undissociated compounds, in contrast, give only one particle in solution, which exerts osmotic pressure.

For example :

a. *Substance that does not dissociate (give one particle in solution)*

(*i*) Glucose (molecular wt. = 180)

$$1 \text{ Osm} = \frac{180 \text{ g}}{1} = 180 \text{ g}$$

$$1 \text{ mOsm} = \frac{180 \text{ mg}}{1} = 180 \text{ mg}$$

(*ii*) Sodium (Na^+; molecular wt. = 23)

$$1 \text{ Osm} = \frac{23 \text{ g}}{1} = 23 \text{ g}$$

$$1 \text{ mOsm} = \frac{23 \text{ mg}}{1} = 23 \text{ mg}$$

(*iii*) Magnesium (Mg^{++}, molecular wt. = 24.5)

$$1 \text{ Osm} = \frac{24.5 \text{ g}}{1} = 24.5 \text{ g}$$

$$1 \text{ mOsm} = \frac{24.5 \text{ mg}}{1} = 24.5 \text{ mg}$$

Therefore for undissociated molecule, 1 mole (1 Mol) = 1 Osm, and 1 millimole = 1 mOsm.

b. *Substance that dissociates (give two, three, or more particles in solution)*

(*i*) Sodium chloride (NaCl; molecular wt. = 58.5)

As sodium chloride in solution gives two particle i.e. Na^+ and Cl^-, the number of Osm or mOsm will be

$$1 \text{ Osm} = \frac{58.5 \text{ g}}{2} = 29.25 \text{ g}$$

$$1 \text{ mOsm} = \frac{58.5 \text{ mg}}{1} = 29.25 \text{ mg}$$

Therefore for molecules which dissociate into 2 particles in the solution, 1 mole (1 Mol) = 2 Osm, and 1 millimole = 2 mOsm.

Although the expression osmolality more correctly describes the osmotic properties, in physiological studies (*in vivo*) it is more practical to measure osmolarity. As the differences between the two expressions are small, these two terms can be used almost synonymously or interchangeably.

III. TONICITY

The term tonicity refers to ability of a solution to initiate water movement across a semi-permeable membrane. It is commonly used when describing the response of cells immersed in an external solution. Like osmotic pressure, tonicity is influenced only by solutes which cannot cross the membrane (impermeant solutes). In such conditions, if the number of impermeant particles on one side of membrane increases or decreases, water either enters or leaves that compartment to maintain the osmolality. Thus tonicity may be thought of as 'effective osmolality'. On the other hand, solutes able to freely cross the membrane (e.g. urea and ethanol) contribute to osmolality, but do not affect tonicity because they will always be in equal concentrations on both sides of the membrane. Therefore, such permeant particles can produce changes in osmolality of the ECF but do not exert tonic pressure.

Examples of particles exerting tonic pressure include sodium chloride, glucose and albumin. If sodium is added to the ECF, it stays there (sodium can diffuse across cellular membranes into intracellular compartments but Na^+-K^+- ATPase pushes it back into ECF) and increases the osmolality and tonicity. Consequently water is drawn out of the ICF and pushed into the ECF until the osmolality of two fluids compartments are equal. Chloride atoms usually follow sodium to maintain electrical neutrality. Albumin contributes most to tonicity at the level of capillaries, where it exerts a tonic force, drawing fluid back into capillaries. Highly permeating substances (e.g. urea) can cause transient shifts in fluid volumes between the intracellular and extracellular fluids, but after some time the concentrations of these substances become equal in two compartments. Such substances have little effect on intracellular volume under steady state conditions.

Types of solutions

There are three types of solutions in relation to tonicity: isotonic, hypotonic and hypertonic.

Isotonic solution: It is the property of a solution in which its solute concentration is the same as the solute concentration of another solution with which it is compared. *In vivo*, an isotonic solution may be defined as a solution that exerts osmotic pressure approximately equal to that of circulating fluid or ECF, so that water neither enters nor leaves the intracellular compartment.

If a cell is placed in an isotonic solution, it will neither shrink nor swell because the water concentrations in the intracellular and extracellular fluids are equal and the solutes neither enter nor leave the cell. Isotonic solutions having impermeant solutes have an osmolarity of approximately 300 mOsm/L (280 – 330 mOsm/L).

Examples of isotonic solutions are 0.9% sodium chloride solution (normal saline) and 5% dextrose solution.

(*i*) Sodium chloride (molecular wt = 58.5; number of particles in solution = 2)

$$1 \text{ mOsm} = \frac{58.5 \text{ mg}}{2} = 29.25 \text{ mg}$$

Isotonic solution (300 mOsm/L)

$= 300 \times 29.25 \text{ mg} = 8775 \text{ mg/L}$

$= 877.5 \text{ mg/100 ml} = 0.8775\%$

$\approx 0.88\%$ or 0.9%

The value of 0.9% w/v is an approximation that also accommodates non-ionic behaviour of NaCl i.e. 100% dissociation of sodium chloride does not occur.

(*ii*) Glucose/Dextrose (molecular wt = 180; number of particles in solution = 1)

$$1 \text{ mOsm} = \frac{180 \text{ mg}}{1} = 180 \text{ mg}$$

Isotonic solution (300 mOsm/L)

$= 300 \times 180 \text{ mg} = 54000 \text{ mg/L}$

$= 5400 \text{ mg/100 ml} = 5.4\% \approx 5\%$

Hypotonic Solution: A hypotonic solution is a solution that exerts osmotic pressure lesser than that of isotonic solution or it is a solution that has osmotic pressure lesser than that of circulating fluid or ECF. This is because it contains a lesser concentration of impermeable solutes than the circulating fluid or ECF.

If a cell is placed into a hypotonic solution, water will diffuse into the cell causing it to swell because water concentration outside the cell is more than that inside the cell. Water will continue to diffuse into the cell until both solutions have about the same osmolality. Hypotonic solutions having impermeant solutes have an osmolarity less than 300 mOsm/L. Solutions of sodium chloride with a concentration of less than 0.9% are hypotonic and cause cells to swell.

Hypertonic Solution: A Hypertonic solution is a solution that exerts osmotic pressure greater than that of isotonic solution, or it is a solution that has osmotic pressure greater than that of circulating fluid or ECF. This is because it contains a greater concentration of impermeable solutes than the circulating fluid or ECF.

If a cell is placed into a hypertonic solution, water will diffuse out of the cell into the extracellular fluid, causing the cell to shrink. Hypertonic solutions having impermeant solutes have an osmolarity greater than 300 mOsm/L. Sodium chloride solutions of concentration greater than 0.9% are hypertonic.

Isosmotic solution: It may be defined as a solution having the same osmotic pressure as the blood (or any other physiological solution) regardless of whether the solute permeates the cell membrane.

Hypo-osmotic solution: It is a solution that exerts osmotic pressure lesser than that of isosmotic solution, regardless of whether the solute permeates the cell membrane. The hypo-osmotic solution usually contains a lower concentration the total amount of osmotically active solutes (both permeable and impermeable) than the isosmotic solution.

Hyper-osmotic solution: It is a solution that exerts osmotic pressure greater than that of isosmotic solution, regardless of whether the solute permeates the cell membrane. The hyper-osmotic solution usually contains a higher concentration of the total amount of osmotically active solutes (both permeable and impermeable) than the isosmotic solution.

In kidneys, the tubular fluid is iso-osmotic (to plasma) when it is at the beginning of the loop of henle, hyper-osmotic (to plasma) when it is at the tip of the loop, and hypo-osmotic (to plasma) when it leaves the loop.

IV. HYDROGEN ION CONCENTRATION AND pH

The concentration of hydrogen ion in an aqueous solution normally determines its acidity or basicity. Because hydrogen ion concentration in various solutions and body fluids is low and these small numbers are cumbersome (see Table 38.2), it is customarily expressed in pH units.

pH

The pH is defined as a negative logarithm of the hydrogen ion concentration in a solution.

$$\text{pH} = \log \frac{1}{[H^+]} = -\log [H^+]$$

For example :

The normal hydrogen ion concentration $[H^+]$ in arterial blood is 40 nEq/L (i.e. 0.00000004 Eq/L). Therefore, the normal pH of arterial blood will be

$$\text{pH} = -\log [0.00000004] = 7.4$$

The concept of pH was first introduced by Danish chemist Søren Peder Lauritz Sørensen at the Carlsberg Laboratory in 1909. Although not exactly known, it is suggested that the abbreviation pH stands for the Latin terms *pondus Hydrogenii* or *potentia hydrogenii*, the power or potential of hydrogen ion. In chemistry, pH is commonly used as a measure of the acidity or basicity of an aqueous solution.

pH Scale: The pH scale, (0 - 14), is the full set of pH numbers which indicate the concentration of H^+ in water. Thus pH scale measures the acidity or basicity of a substance. In the

Table 38.2: H^+ ion concentration and pH of body fluids.

Body Fluid	H^+ Concentration (mEq/L)	pH
1. Extracellular Fluid		
a. Arterial blood	4.0×10^{-5}	7.40
b. Venous blood	4.5×10^{-5}	7.35
c. Interstitial blood	4.5×10^{-5}	7.35
2. Intracellular Fluid	1×10^{-3} to 4×10^{-5}	6.0 to 7.4
3. Urine	3×10^{-2} to 1×10^{-5}	4.5 to 8.0
4. Gastric HCl	160	0.8

scale, H^+ ion concentration and pH relate inversely i.e. increasing pH means the H^+ ions are decreasing, and decreasing pH means H^+ ions are increasing. A pH of 7 is neutral, a pH less than 7 is acidic and a pH greater than 7 is basic. The pH scale is logarithmic and as a result, each whole pH value below 7 is ten times more acidic than the next higher value. For example, pH 4 is ten times more acidic than pH 5 and 100 times more acidic than pH 6. The same holds true for pH values above 7, each of which is ten times more alkaline.

Physiological significance

The pH of different cellular compartments, body fluids and organs is usually tightly regulated in a process called acid-base homeostasis. The pH of blood is slightly basic with a value of pH 7.365 (~7.4). This value is often referred to as physiological pH in biology and medicine. An increase or decrease in the blood pH results in alkalosis or acidosis, a common disorder in acid-base homeostasis.

Buffer solution

Buffer solution is an aqueous solution that can resist changes of pH upon addition of an acid or base. A buffer system consists of a weekly-dissociated acid and the salt of the acid. The buffers are able to take up or release hydrogen ion so that changes in hydrogen ion concentration are minimised.

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CHAPTER 39 DIURETICS

Classification
High efficacy diuretics
Loop diuretics/High ceiling diuretics
Mechanism of action
Mercurials
Moderate efficacy diuretics
Thiazides
Mechanism of action
Thiazide-like diuretics
Low efficacy diuretics/Adjunctive diuretics
Osmotic diuretics
Mechanism of action
Carbonic anhydrase inhibitors
Mechanism of action
Potassium sparing diuretics
Inhibitors of renal epithelial Na^+ channels
Mechanism of action
Aldosterone receptor antagonists
Mechanism of action
Miscellaneous diuretics
Acidifying or alkalinising salts/Saline diuretics
Acidifying salts/Urinary acidifiers
Alkalinising salts
Methylxanthines/Xanthine derivatives
Newer drugs
Aquaretics
Vaptans/Vasopressin receptor antagonists
Mechanism of action
Dopamine receptor agonists

Diuretics are commonly defined as drugs which increase the amount of urine produced by kidneys. More precisely, diuretics may be defined as agents which promote a net loss of sodium ions (natriuresis) and accompanying Cl^- and HCO_3^- ions and water from body resulting in an increase in the rate of urine formation. Substances which do not have primary action on the kidney such as ethanol (acts by inhibition of ADH secretion from supraoptic nuclei) and water (acts by decreasing osmotic pressure of plasma) are generally not considered as diuretics since they increase the output of water without any loss of electrolytes. Similarly, digitalis that promotes urine flow by improving cardiac output in congestive heart failure (CHF) and has no direct action on the normal kidney is also not regarded as a diuretic.

In veterinary medicine diuretics are mainly used to increase urine flow when there is fluid retention and oedema in cases of heart failure, hepatic disease, cerebral oedema and hypoproteinaemia. These are also useful to induce urine flow in renal failure, to create forced excretion of poisons or drugs in overdose, and to reduce systemic blood pressure. Diuretics are the mainstay of therapy for CHF where there is systemic or pulmonary fluid retention. Most diuretic drugs increase urine volume by reducing the efficiency of sodium reabsorbing processes and thus increasing the obligatory water loss. Diuretics alter not only the excretion of Na^+, but may also renal transport of certain other cations (e.g. K^+, H^+, Ca^{++} and Mg^{++}), anions (Cl^-, HCO_3^- and $H_2PO_4^-$) and uric acid.

Classification

Diuretics have been classified in various ways such as according to their major site of action, mechanism of action, chemical structure, potency, or alterations of urine pH. According to their potency, diuretics are classified into following groups:

I. High efficacy diuretics
   1. High ceiling diuretics/Loop diuretics
      e.g. furosemide, etacrynic acid and bumetanide.
   2. Mercurial/Organomercurial diuretics
      e.g. mersalyl.

II. Moderate efficacy diuretics
   1. Thiazides
      e.g. chlorothiazide, hydrochlorothiazide, bendroflumethiazide and polythiazide.
   2. Thiazide-like diuretics
      e.g. chlortalidone, clopamide, metolazone and xipamide.

III. Low efficacy or adjunctive diuretics
   1. Osmotic diuretics
      e.g. mannitol, glycerine, sorbitol and isosorbide.
   2. Carbonic anhydrase inhibitors
      e.g. acetazolamide, dichlorphenamide, ethoxzolamide and methazolamide.
   3. Potassium-sparing diuretics
      a. Inhibitors of renal epithelial Na^+ channels
         e.g. triamterene, amiloride and benzamil.
      b. Aldosterone receptor antagonists
         e.g. spironolactone, eplerenone, canrenone and potassium canrenoate.

IV. Miscellaneous diuretics
   1. Acidifying or alkalinising salts
      a. Acidifying salts
         e.g. ammonium chloride and sodium chloride.
      b. Alkalinising salts
         e.g. potassium acetate and potassium citrate.
   2. Methylxanthines/Xanthine diuretics
      e.g. aminophylline and theophylline.
V. Newer drugs
   1. Aquarectics
      a. Vaptans/ Vasopressin receptor antagonists
         e.g. conivaptan, tolvaptan and mozavaptan.
   2. Dopamine receptor agonists
      e.g. fenoldopam.

I. HIGH EFFICACY DIURETICS

1. Loop Diuretics/ High Ceiling Diuretics

Loop diuretics (High ceiling diuretics/Inhibitors of Na^+-K^+-$2Cl^-$ Symport) are chemically diverse groups of drugs which have in common an ability to block the Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of loop of Henle (hence called loop diuretics). They are the most potent group of diuretics with maximal natriuretic effect up to 30% of the filtered load of NaCl and water, much greater than any other class of diuretics. For this reason, they are often called high-ceiling diuretics. Because of their high potency, excessive doses of loop diuretics can lead to hypovolaemia and decompensation of renal function. However, their potency allows them to remain effective even when urine delivery is poor as in renal impairment.

Mechanism of Action

Loop diuretics bind to the Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb and block its functioning, stopping salt transport completely in this segment of the nephron (Figure 38.4). The molecular mechanisms by which loop diuretics block the Na^+-K^+-$2Cl^-$ symporter is not clear, but evidence suggests that these drugs attach to the Cl^- binding site of symporter. A minor component of action on proximal tubule and distal tubule has also been identified. Loop diuretics also block Ca^{++} and Mg^{++} reabsorption in the thick ascending limb by abolishing transepithelial potential difference that is the dominant driving force for their reabsorption. Prolonged use of loop diuretics can cause significant hypomagnesemia in some patients. Since Ca^{2+} is actively reabsorbed in the distal convoluted tubule, loop diuretics do not generally cause hypocalcaemia.

The high efficacy of loop diuretics is due to their ability to block about 25-30% of the filtered solute load normally absorbed via Na^+-K^+-$2Cl^-$ symporter. Although the proximal tubule is responsible for reabsorption of most of the filtered solutes (>65%), diuretics acting only in the proximal tubule has low efficacy because the rejectate from proximal tubule are absorbed efficiently through the ascending limb of loop of Henle. In contrast, inhibition of only 25-30% of filtrate by loop diuretics in the thick ascending limb has marked influence on diuresis because nephron segments past the thick ascending limb (distal tubules and collecting ducts) do not posses sufficient resorption capacity (<5%) and cannot reabosrb most of the rejectate of thick ascending limb of loop of Henle.

Furosemide

Furosemide (Frusemide) is a loop diuretic that is related structurally to the sulphonamides (therefore also called sulphonamide loop diuretic). Furosemide contains a free carboxyl group that helps it to be transported into a longer segment of the tubule after its excretion in the proximal tubule. Hence it inhibits sodium and chloride reabsorption over a longer length of the nephron producing rapid diuretic effect by inhibiting Na^+-K^+-$2Cl^-$ symporter. As furosemide possesses a sulphonamide moiety, it is also a week inhibitor of carbonic anhydrase enzyme, a property not shared by non-sulphonamide loop diuretics (e.g. etacrynic acid). Additionally, furosemide is a noncompetitive subtype-specific blocker of $GABA_A$ receptors.

Pharmacological effects

Furosemide causes a profound increase in the urinary excretion of Na^+ and Cl^- due to effective blockade of the Na^+-K^+-$2Cl^-$ symporter. The K^+ and H^+ excretion are also enhanced mainly due to high Na^+ load reaching distal tubule. In addition, it increases Ca^{++} excretion (contrast thiazides which reduce it) as well as Mg^{++} excretion. It tends to raise blood uric acid level by decreasing its renal excretion. This is due to interference with tubular secretion of uric acid and increased reabsorption in proximal tubule, which is a consequence of reduced extra cellular volume fluid. However, the magnitude of hyperuricaemia is lower than that of thiazides. Furosemide has week carbonic anhydrase inhibitory action and increases excretion of HCO_3^- as well. However, its action is independent of the acid-base balance of body and it causes minimum distortion of the same; mild alkalosis may occur at high doses. Overall furosemide increases renal excretion of water, sodium, chloride (most affected), potassium, calcium, magnesium, hydrogen, ammonium and bicarbonate.

In addition to its prominent tubular action, furosemide causes acute changes in renal and systemic haemodynamics. Furosemide may decrease peripheral resistance and cause increase in renal blood flow, renal vasodilatation and transient increase in glomerular filtration rate. It increases systemic venous capacitance and thereby decreases left ventricular filling pressure. The intrarenal haemodynamic changes are brought about by increased local prostaglandins synthesis. Furosemide can cause hyperglycaemia, but to a lesser extent than that of thiazides.

Pharmacokinetics

Furosemide is rapidly adsorbed orally with a bioavailability of about 75% in dogs. The diuretic effect takes place within 5 minutes after IV administration and within one hour after oral dosing. After absorption, the drug is secreted primarily in the proximal tubule by organic anion transport and reaches the ascending limb of loop Henle where it acts from the luminal side of the membrane. It is highly bound to plasma proteins. It is partly conjugated with glucuronic acid and excreted mainly unchanged by glomerular filtration as well as tubular secretion. Some excretion in bile also occurs. Its elimination half-life in dogs is approximately 1-1.5 hours and duration of action 4-6 hours. Furosemide is detected in milk for up to 30 hours after treatment.

Side effects/Adverse effects

Adverse effects of furosemide are mainly related to fluid and electrolyte abnormalities. A rapid reduction in extracellular fluid

volume can result in hypotension and cardiovascular collapse with associated hypokalaemia and hypochloraemic alkalosis. So patients should be monitored closely for hydration status and electrolyte imbalances (especially potassium, sodium and calcium). Extra renal adverse effects related to furosemide treatment include ototoxicity with reversible loss of hearing and tinnitus (especially in cats with high IV dose), gastrointestinal disturbances, haemolytic effects (anaemia, leucopenia and thrombocytopenia), weakness and restlessness. Excessive use of furosemide may lead to a metabolic alkalosis due to hypochloremia and hypokalemia. Overdoses in horses have been associated with dehydration, change in drinking patterns and urination, seizures, GI problems, kidney damage, lethargy, collapse and coma.

Contraindications and precautions

Furosemide is contraindicated in patients with anuria or patients hypersensitive to it. Furosemide should be used with caution in patients with pre-existing electrolyte and water imbalance and impaired hepatic function. Its use during pregnancy or in a lactating mare should be avoided, as it has been shown to be passed through the placenta and milk in studies with other species. It should not be used in horses with pituitary pars intermedia dysfunction (Cushings).

Drug interactions

Furosemide shows interactions with many drugs. Ototoxicity and nephrotoxicity associated with aminoglycoside antibiotics may be increased when used with furosemide. Furosemide induced hypokalaemia may increase the chances of digitalis toxicity. Furosemide enhances the pharmacological effects of theophylline and other xanthines. Concurrent use of NSAIDs may reduce action of furosemide by preventing synthesis of PGs in the kidney. Probenecid when given together with furosemide may interfere with secretion of furosemide and other loop diuretics in the proximal tubule. Cephalosporins usually aggravate kidney damage. Furosemide may not be used concomitantly with purgatives because of excessive fluid and electrolyte loss.

Clinical uses

Furosemide is used for its diuretic activity in all species. It is primarily used to decrease oedema in conditions such as cardiovascular and pulmonary oedema, hepatic and renal dysfunction, hydrothorax, ascites and non-specific oedema. The drug is used in thorough bred and harness race horses, where it is believed to diminish the incidence of epistaxis by reducing central venous pressure. Furosemide is also useful when an immediate diuresis is indicated to control the fluid overload or when the glomerular filtration rate is markedly reduced.

Along with some other diuretics, use of furosemide during sporting events is banned due to its alleged use as a masking agent for other drugs.

Dose

As general diuretic for oedema

Dogs & cats : 2 – 4 mg/kg, PO, IV or IM, 1 to 2 times daily. Dose should be reduced to 1 – 2 mg/kg, 1 to 2 times daily for maintenance.

Cattle : 2 – 5 mg/kg, PO.

: 0.5 – 1 mg/kg, IM or IV.

Pigs : 5 mg/kg, IM or IV.

Horses : 0.5 – 1 mg/kg, PO, IM or IV, 1 to 2 times daily.

Birds : 0.05 mg/300 g body weight, IM, 2 times daily.

For acute renal failure

Dogs & cats : 2 mg/kg, IV, after replacing fluid deficit. If no diuresis occurs within 1 hour, administer 4 mg/kg, IV. If no response in 1 hour, give another dose of 6 mg/kg, IV.

For hypercalcaemia/Hypercalcuric nephropathy.

Dogs & cats : 5 mg/kg, bolus IV followed by 5 mg/kg/hour IV infusion. Hydration status and electrolyte balance should be maintained with normal saline with KCl added.

For epistaxis prevention

Horses : 0.5 mg/kg, IM or IV, 60 to 90 minutes before race.

Etacrynic acid

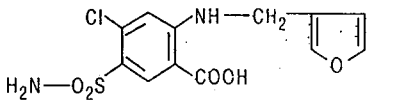
Etacrynic acid (Ethacrynic acid) is a non-sulphonamide loop diuretic that shares the same properties and indications as furosemide. Etacrynic acid forms a cysteine adduct with methylene group in the body, which is considered the active form. Similar to furosemide, etacrynic acid acts by inhibiting the Na^+-K^+-2Cl symporter in the ascending limb of loop of Henle. However, due to a different chemical structure, it does not possess the week inhibitory action on carbonic anhydrase enzyme, so there is no increase in HCO_3^- excretion. Loss of K^+ is less marked but chances of hypochloraemic alkalosis are greater than furosemide. Chances of ototoxicity due to alterations in the electrolyte composition of endolymph are more with etacrynic acid. Etacrynic acid and its glutathione-adduct are potent inhibitors of glutathione S-transferase family members, which are enzymes involved in xenobiotic metabolism.

Dose

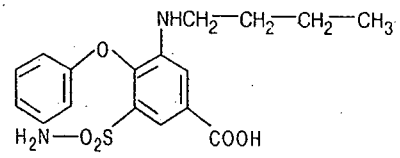
Dogs & cats: 0.2 – 0.4 mg/kg, IM or IV, 2 to 4 times daily.

Bumetanide

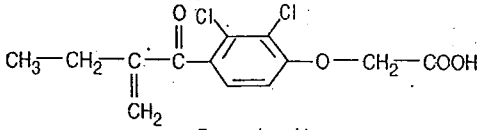
Bumetanide is a sulphonamide loop diuretic that is similar to furosemide in all respects. It is about 40 times more potent than furosemide and induces very rapid diuresis. It may also be considered useful in some cases not responding to furosemide.



Furosemide



Bumetanide



Etacrynic acid

Fig. 39.1. Chemical structures of selected high ceiling (loop) diuretics.

Bumetanide is well absorbed (>80%) after oral administration and its absorption is not affected by presence of food in the GI tract.

Other Drugs

Several other sulphonamide and non-sulphonamide loop diuretics are currently available in human medicine. These include azosemide, piretanide, tripamide (all sulphonamide derivatives), mezolimine and torsemide (non-sulphonamide diuretics).

2. Mercurials

The mercurials (Organomercurials) were choice diuretics for many years whenever a prompt and potent diuresis was indicated. These included mersalyl (mersalyl acid), meralluride, mercaptomerin and chlormerodin. In past decades, several new groups of diuretics were introduced, which have almost replaced organomercurials.

In acidic pH, organomercurial drugs liberate mercuric ion that combines with -SH group of enzymes associated with transport system in the kidney tubule. These drugs depress mainly the reabsorption of Na^+ and Cl^- with less effect on K^+ and HCO_3^-. It is suggested that their primary action is on Na^+-K^+-2 Cl^- symporter in ascending limb of loop of Henle. Production of diuresis causes a hypochloraemic alkalosis (due to excessive secretion of Cl^- but retention of HCO_3^-), which then suppresses the pH dependent efficacy of mercurial diuretics. When acidifying agents (e.g. ammonium chloride) are given, the diuretic effect of mercurial is restored.

Mercurial diuretics are highly toxic compounds. They may produce systemic mercury poisoning and attenuate kidney damage. Further, they are effective only when administered parentally. Due to these reasons, the mercurial diuretics are seldom used therapeutically. Because the mercurial diuretics do not disrupt K^+ balance as much as many other diuretics they occasionally are used in CHF, cirrhosis and portal obstruction. Therapeutically, mercurial diuretics are effective when used only intermittently since regular administration causes alkalosis and refractoriness.

II. MODERATE EFFICACY DIURETICS

1. Thiazides

Thiazides (Na^+-Cl^- symport inhibitors) are sulphonamide derivatives and are related in structure to the carbonic anhydrase inhibitors. They primarily act as inhibitors of Na^+-Cl^- co-transport and have significantly greater diuretic activity than structurally related carbonic anhydrase inhibitors. Most of these diuretics are derived from benzothiadiazine, a bicyclic heterocyclic benzene derivative with the heterocycle containing two nitrogens and one sulphur, so this class of diuretics is commonly known as thiazide diuretics.

Mechanism of Action

The thiazide diuretics act mainly in the distal tubule to decrease the reabsorption of Na^+ by inhibition of the Na^+-Cl^- co-transporter in the luminal membrane (Figure 38.5). In addition, they have a secondary effect in the proximal tubule. However, they do not have any action on the medullary thick ascending limb of loop Henle. These drugs gain access to their site of action primarily via the organic acid secretory pathway in the proximal tubule and then along tubular fluid to the early distal tubule, where they bind to specific Na^+-Cl^- symporter. Some of the drugs have in addition carbonic anhydrase inhibitory action in proximal tubule. The intensity of carbonic anhydrase inhibitory action is variable among different compounds, but is generally weak and clinically insignificant.

The thiazide diuretics are moderately efficacious diuretics causing excretion of about 5% of sodium in the filtrate because nearly 90% of the glomerular filtrate has already been reabsorbed before it reaches the distal convoluted tubule. The thiazides do not cause significant alteration in acid-base balance and their diuretic effectiveness is independent of acid-base alterations.

Chlorothiazide

Chlorothiazide sodium is the prototype thiazide diuretic that is structurally related to the sulphonamides. It was first introduced as a carbonic anhydrase inhibitor, but primarily its mode of action is inhibition of Na^+-Cl^- symport in the distal convoluted tubule.

Pharmacological effects

Chlorothiazide inhibits reabsorption of Na^+ and Cl^- in the distal convoluted tubule, which results in enhanced renal excretion of Na^+ and Cl^-, and also HCO_3^- and K^+. The excretion of K^+ in urine is due to enhanced delivery of Na^+ to the distal nephron where it is exchanged for K^+. Thus prolonged use of this drug causes continual loss of K^+ from the body. The loss of HCO_3^- is due to inhibition of carbonic anhydrase enzyme by chlorothiazide. Chlorothiazide decreases the Ca^{++} content of urine by promoting its reabsorption. This contrasts with the loop diuretics which increase the Ca^{++} concentration in the urine. Chlorothiazide occupies the uric acid secretory transport mechanism and decreases also uric acid excretion. Long-term use of chlorothiazide may cause magnesium deficiency by causing mild magnesuria by a poorly understood mechanism. In general, chlorothiazide has no significant primary effect on acid-base balance and its diuretic effect is independent of acid-base alteration. Refractoriness does not occur due to its diuretic effect.

The extra-renal actions of chlorothiazide consists of a slowly developing fall in blood pressure in hypertensive animals resulting from reduced peripheral resistance caused by direct relaxation of arteriolar smooth muscles. Reduction in blood pressure also results from a decrease in blood volume and, therefore, a decrease in cardiac output. Chlorothiazide does not markedly affect the renal blood flow and only variably reduce glomerular filtration rate. Although chlorothiazide is a diuretic, it causes a significant reduction in urine volume in patients of diabetes insipidus. The mechanism of this paradoxical antidiuretic effect is not clearly understood.

Pharmacokinetics

Like other thiazides, chlorothiazide is well absorbed when given orally and is excreted in the proximal tubule by kidney by organic acid secretory system. Chlorothiazide is distributed throughout the extracellular space and does not accumulate in tissues other than the kidneys. In humans, the onset of diuretic activity occurs within 1-2 hours, peaks at 4 hours and lasts for 8-12 hours.

Side effects/Adverse effects

Chlorothiazide has relatively few side effects and has wide margin of safety. Hypokalaemia is one of the most common adverse effects, but it rarely causes symptoms. Hypochloraemic alkalosis (with hypokalaemia) may develop especially if there are other causes of potassium and chloride loss (e.g. vomiting and

diarrhoea). Hyperuricaemia may precipitate existing gout, while hyperglycaemia may aggravate diabetes mellitus. It may rarely cause sulphonamide type skin reactions, leucopenia, agranulocytosis and hypersensitivity reactions. Acute over dosage may lead to electrolyte and water imbalance. Central nervous system effects (seizures) and GI effects (diarrhoea, GI distress and vomiting) may also be seen.

Contraindications and precautions

Chlorothiazide and other thiazides should not be used in renal failure with anuria. These are also contraindicated in patients hypersensitive to sulphonamides. Chlorothiazide should be used with extreme care in patients with pre-existing electrolyte or water abnormalities, impaired hepatic function, hyperuricaemia or diabetes mellitus. Thiazides can decrease placental perfusion and adversely affect the foetus so should be avoided in pregnancy.

Drug interactions

Chlorothiazide induced hyperkalaemia may increase the likelihood of digitalis toxicity. Sulphonamides may potentiate chlorothiazide activity.

Clinical uses

Chlorothiazide is used to treat cardiac or hypoproteinaemiac oedema. Paradoxically, it has also been used in the treatment of diabetes insipidus, where it decreases the polyuria, especially in patients those cannot tolerate ADH or do not respond to ADH. It is useful in the management of hypercalciuria in dogs where it helps to prevent recurrence of calcium oxide uroliths. Chlorothiazide is approved for use in dairy cattle for the treatment of post-parturient udder oedema. Occasionally it is used in the treatment of hypertension.

Dose

For treatment of oedema (as diuretic)

Dogs & cats : 20 – 40 mg/kg, PO, 2 times daily.

Cattle : 4 – 8 mg/kg, PO, 1 to 2 times daily.

For nephrogenic diabetes insipidus

Dogs & cats : 20 – 40 mg/kg, PO, 2 times daily.

For systemic hypertension

Dogs : 20 – 40 mg/kg, PO, 1 to 2 times daily with salt restriction.

Hydrochlorothiazide

Hydrochlorothiazide is a thiazide diuretic that is closely related to chlorothiazide. It is also one of the commonly used diuretic of this group. The pharmacological properties, adverse effects and therapeutic uses of hydrochlorothiazide are related to chlorothiazide. The onset of diuretic activity occurs in 2 hours after its oral administration, but the effect persists for comparatively longer period (up to 12 hours) than chlorothiazide. On equal weight basis, hydrochlorothiazide is 10 to 20 times more potent than chlorothiazide.

Dose

For treatment of oedema (as diuretic)

Dogs & cats : 2 – 4 mg/kg, PO, 1 to 2 times daily.

: 12.5 – 25 mg (total), IM.

Horses & cattle: 500 mg (total), PO initially followed by 250 mg (total), PO, 1 to 2 times daily.

: 100 – 250 mg (total), IV or IM.

For udder oedema

Cattle : 125 – 250 mg (total), IV or IM, 1 to 2 times daily. Drug may be continued for a few days, if necessary.

For treatment of nephrogenic diabetes insipidus

Dogs : 0.5 – 2 mg/kg, PO, 2 times daily.

For systemic hypertension

Dogs : 2 – 4 mg/kg, PO, 1 to 2 times daily with salt restriction.

For treatment of recurrent calcium oxalate uroliths with renal hypercalciuria

Dogs : 2 mg/kg, PO, 2 times daily.

Bendroflumethiazide

Bendroflumethiazide (Bendrofluazide) is another thiazide diuretic having properties and actions similar to chlorothiazide. However, it is more potent and longer acting diuretic that is effective for up to 24 hours

Dose

Dogs & cats : 0.125 – 0.25 mg/kg, PO, once daily in the morning.

Other drugs

Some other less commonly used thiazide diuretic drugs are cyclopenthiazide, cyclothiazide, hydroflumethiazide, mebutizide, methyclothiazide, polythiazide and trichlormethiazide

2. Thiazide-like Diuretics

Thiazide-like diuretics are a recently discovered group of sulphonamide diuretic drugs which possess pharmacological properties similar to a thiazide diuretic, but do not have the chemical properties of a thiazide, lacking the benzothiadiazine molecular structure. The primary target of all non-thiazide diuretics is the distal convoluted tubule, where they inhibit the sodium-chloride symporter. Since these drugs act on the same target as thiazides and behave in a similar pharmacological fashion, they are often discussed along with thiazide diuretics.

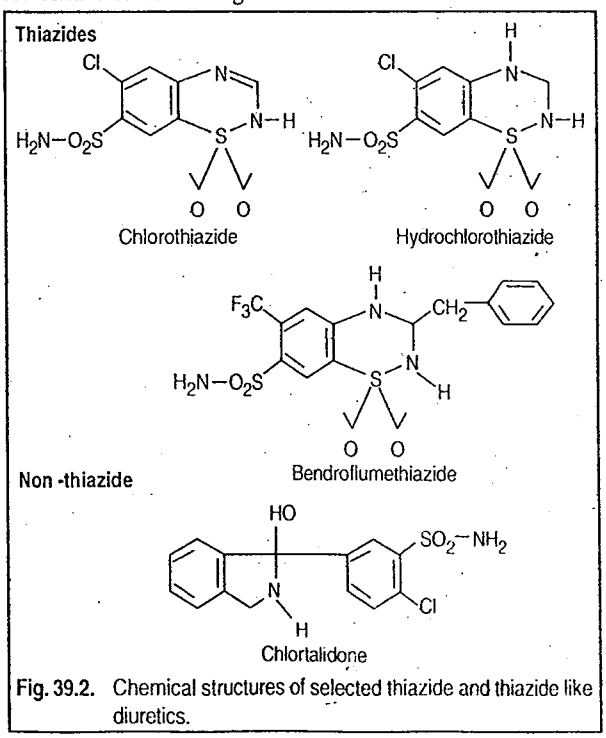


Fig. 39.2. Chemical structures of selected thiazide and thiazide like diuretics.

Chlortalidone

Chlortalidone (Chlorthalidone) is the prototype thiazide-like (non-thiazide) diuretic. Its mechanism of action and pharmacological properties are similar to thiazide diuretics (e.g. chlorothiazide); however, it does not contain the benzothiadiazine molecular structure present in thiazide diuretics. Chlortalidone is approximately equal in its diuretic effect to maximal therapeutic doses of benzothiadiazine diuretics. The site of action appears to be the cortical diluting segment of the ascending limb of Henle.

In human beings, chlortalidone is slowly absorbed and slowly excreted from body as a result the diuretic effect of the drug occurs in approximately 2.6 hours and continues for up to 72 hours. Approximately 75 percent of the drug is bound to plasma proteins. The drug also has increased affinity for the erythrocyte carbonic anhydrase. The major portion of the drug is excreted unchanged by the kidneys. Chlortalidone is indicated mainly in human medicine in the management of hypertension either as the sole therapeutic agent or to enhance the effect of other antihypertensive drugs in the more severe forms of hypertension. It is also indicated as adjunctive therapy in oedema associated with congestive heart failure, hepatic cirrhosis, and corticosteroid and oestrogen therapy.

Other drugs

Several other non-thiazide diuretics are available for use in human medicine, which have properties and actions similar to those of chlortalidone. Apart from the differences in onset of action and duration of action, there is no inherent superiority of one compound over the other. These include clopamide, clofenamide, clorexolone, fenquizone, indapamide, mefruside, meticrane, metolazone, quinethazone and xipamide.

III. LOW EFFICACY DIURETICS/ADJUNCTIVE DIURETICS

1. Osmotic Diuretics

Osmotic diuretics are pharmacologically inert non-electrolyte substances, which are freely filtered at the glomerulus but not significantly reabsorbed from the tubules. Osmotic diuretics are administered in large doses to increase significantly the osmolality of plasma and tubular fluid.

Mechanism of action

Osmotic diuretics appear to limit tubular reabsorption of water and electrolyte in a variety of ways:

a. Osmotic diuretics inhibit passive reabsorption of water in those segments of nephron which are freely permeable to water i.e. the proximal tubule, descending limb of loop of Henle and also distal tubule. Under normal conditions, when Na^+ and Cl^- are reabsorbed there is passive back diffusion of water to keep the tubular fluid isosmotic. However in the presence of osmotic diuretic, the overall osmotic relationship within nephron remains almost unaltered and tubular fluid is isosmotic with plasma. Thus back diffusion of water does not take place even when Na^+ and Cl^- are reabsorbed. Therefore, the main effect of osmotic diuretics is to increase the amount of water excreted with a relatively smaller increase in sodium excretion.

b. Osmotic diuretics extract water from intracellular compartments and expand extracellular fluid volume. This decreases blood tonicity, increases renal blood flow and glomerular filtration rate, and reduces renin and aldosterone secretion. This limits Na^+ and water reabsorption.

c. The increase in renal medullary blood flow removes excess of Na^+, Cl^- and urea from the renal medulla, thus reducing medullary tonicity. A reduction in medullary tonicity causes a decrease in the extraction of water from the descending limb of loop of Henle and subsequently a passive salt reabsorption from the ascending thin loop of Henle.

d. It is suggested that osmotic diuretics also inhibit transport processes in thick ascending limb of loop of Henle by an unknown mechanism. The significance for this action is still not much clear.

Mannitol

Mannitol is non-electrolyte and pharmacologically inert six carbon sugar alcohol. It is freely filtered at the glomerulus and undergoes negligible reabsorption from the renal tubules, which prevents reabsorption of water by osmotic action. Mannitol is used as an osmotic diuretic agent and a weak renal vasodilator.

Pharmacological effects

Administration of mannitol causes water retention within the nephron causing its enhanced excretion. Though the primary action of mannitol is to increase urinary volume, but excretion of all cations and anions is also enhanced. In general, it increases the urinary excretion of Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, HCO_3^- and phosphate. It also enhances excretion of urea and uric acid in the urine. Mannitol may have a nephroprotective effect, as it is able to maintain the urine volume even when the GFR is reduced. In addition, it does not allow accumulation of nephrotoxins in the renal tubule due to its water retention properties. Like other osmotic diuretics, mannitol may increase renal blood flow and glomerular filtration by causing renal arteriolar dilation, decreased vascular resistance and reduced blood viscosity.

Extra-renal effects of mannitol include reduction in intraocular and CSF pressure through its osmotic effects. By elevating the osmolality of plasma, it enhances the diffusion of water from these sites (e.g. CSF) back into the plasma.

Pharmacokinetics

Mannitol is poorly absorbed from GI tract, so it has to be given intravenously as 10-20% solution. After IV administration, mannitol is distributed to extracellular fluid and does not penetrate the eye or CNS. Less than 10% of the administered dose is metabolised; the remainder is excreted unchanged in urine. Half-lives of mannitol in cattle and sheep are reported to be between 40-60 minutes.

Side effects/Adverse effects

Mannitol causes fluid and electrolyte imbalance. It increases blood volume and may precipitate pulmonary oedema, if given in patients of congestive heart failure. Other adverse effects include nausea and vomiting (GI effects), tachycardia and CNS effects. Excessive administration of mannitol can produce severe hypovolaemia with excessive excretion of sodium, potassium and chloride.

Treatment: Mannitol toxicity may be treated by correcting electrolyte and fluid imbalance. Haemodialysis is effective in clearing mannitol.

Contraindications and precautions

Mannitol is contraindicated in patients with anuria secondary to renal disease, severe dehydration, intracranial bleeding and pulmonary oedema. Mannitol therapy should be stopped if progressive renal failure, heart failure and pulmonary congestion develop after its therapy is instituted.

Clinical uses

Mannitol is used to promote diuresis in acute oliguric renal failure. In such abnormal conditions, the GFR is reduced and the absorption of salt and water in proximal convoluted tubule becomes almost complete, so that more distal parts of the nephron virtually dry up and urine flow ceases causing further damage to tubule. Retention of fluid within the proximal tubule by mannitol limits such a renal damage. Mannitol is also used to reduce intracranial pressure in cerebral oedema and cerebral trauma, and intraocular pressure in glaucoma. Mannitol is often used to enhance urinary excretion of drugs and toxins. It is used in conjunction with other diuretics to treat oedema or ascites. Mannitol should always be administered after correcting fluid, electrolyte, acid-base balance and determining that the patient is not anuric.

Dose

As a diuretic for treatment of oliguric renal failure

Dogs	:	500 – 1000 mg/kg, slow IV injection over 15 to 20 minutes as 20-25% solution. Dose may be repeated as necessary (4 to 6 hour intervals), if diuresis occurs.
Cats	:	250 – 500 mg/kg, slow IV. Dose may be repeated as necessary, if diuresis occurs.
Cattle, sheep, goats & swine	:	1000 – 2000 mg/kg, slow IV as 20% solution.
Horses	:	250 – 1000 mg/kg, slow IV or IV infusion as 20% solution. Dose may be repeated as necessary, if diuresis occurs.

For adjunctive treatment of cerebral oedema/increased CSF pressure

Dogs	:	1500 mg/kg, IV, once.
Cattle	:	1000 – 3000 mg/kg, IV (usually with a steroid).

For treatment of acute glaucoma

Dogs	:	1000 – 2000 mg/kg, slow IV over 15-20 minutes. Drinking water should be withheld for about 30 to 60 minutes after administration.

Glycerine

Glycerine (Glycerin, glycerol) is an orally effective osmotic diuretic that occurs as a clear, sweet tasting, syrupy and hygroscopic liquid. It is a trihydric alcohol that is miscible with water and alcohol but not miscible in oils. In therapeutic doses, glycerine increases the osmotic pressure of plasma so that water from extracellular space in drawn into the blood.

Oral glycerine is used primarily for short-term reduction of intraocular pressure and CSF pressure in small animals. Glycerine is less used for diuretic purposes as it is partially metabolised by the liver before excretion in the urine. Vomiting after dosing is the most common adverse effects seen with glycerine use.

Other drugs

Several sugars and sugar alcohols like glucose, sucrose, sorbitol and isosorbide may be used in hypertonic concentrations for their osmotic effect. These agents also increase plasma osmolality on IV administration and may be beneficial in the treatment of increased intraocular pressure. Urea, if used intravenously, also tends to draw fluid from the extracellular space into the blood. However, urea is more toxic and is contraindicated in patients with impaired hepatic, cardiac and renal functions.

2. Carbonic Anhydrase Inhibitors

Carbonic anhydrase (CA) inhibitors are a group of drugs which suppress the activity of carbonic anhydrase enzyme, which catalyses the first part of a reversible reaction, in which carbon dioxide (CO_2) and water (H_2O) are converted to carbonic acid (H_2CO_3) and vice-versa. Carbonic anhydrase enzyme thus functions in CO_2 and HCO_3^- transport and in H^+ ion secretion. Carbonic anhydrase inhibitors have limited usefulness as diuretics but have played a major role in the development of fundamental concepts of renal physiology and pharmacology.

Mechanism of action

Carbonic anhydrase enzyme inhibitors block the functioning of carbonic anhydrase enzyme predominantly at the proximal convoluted tubule, reducing the number of hydrogen ions available for Na^+-H^+ exchange. Carbonic anhydrase enzyme in the renal tubule is present in the luminal and basolateral membrane (type IV carbonic anhydrase) as well as in the cytoplasm (type II carbonic anhydrase). Carbonic anhydrase inhibitors block both the membrane bound and cytoplasmic forms of carbonic anhydrase, resulting in diminished excretion of H^+ into the lumen and nearly complete abolition of Na^+ and HCO_3^- reabsorption in the proximal tubule and also in the collecting duct system. Because of decreased Na^+ reabsorption, the Na^+-K^+ exchange in the distal convoluted tubule increases, causing a loss of K^+ in the urine. Among all diuretics, CA inhibitors cause the most marked kaliuresis. The urine produced is alkaline and rich in HCO_3^-.

Acetazolamide

Acetazolamide sodium is the prototype carbonic anhydrase inhibitor. It carries an unsubstituted sulphonamide moiety that is primarily responsible for competitive inhibition of carbonic anhydrase enzyme. Acetazolamide is less used as a diuretic because it is significantly less effective than thiazides and loop diuretics.

Pharmacological effects

The pharmacological effects of acetazolamide include both renal and extra-renal effects. In kidneys, it increases renal tubular secretion of sodium and potassium and, to a greater extent, bicarbonate resulting in an enhanced alkaline urine excretion. To maintain ionic balance, chloride is retained by the kidney resulting in a hyperchloraemic acidosis. The development of metabolic acidosis initiates a compensatory mechanism and moment there is enough H^+ ions for exchange with Na^+, the diuresis ends. The diuretic effect of acetazolamide is thus a self-limiting process. Acetazolamide increases afferent arteriolar resistance and reduces renal blood flow and glomerular filtration rate.

Extra-renal effects of acetazolamide are due to inhibition of carbonic anhydrase enzyme at sites other than renal tubules. This enzyme is present in a number of extra-renal tissues including the eye, gastric mucosa, CNS and red blood cells. Inhibition of CA in the ciliary processes of eye decreases HCO_3^- formation in the

aqueous humours and consequently reduction in intraocular pressure. Inhibition of carbonic anhydrase enzyme in RBCs increases CO_2 level in peripheral tissues and decreases CO_2 levels in expired gas. Acetazolamide raises the seizure threshold to convulsions in the CNS. This anticonvulsant effect of acetazolamide is independent of its diuretic effect and may be due to CA inhibition in the CNS or a metabolic acidosis effect. Large doses of acetazolamide are reported to reduce gastric acid secretion, but this has no therapeutic application.

Pharmacokinetics

Acetazolamide is well absorbed after oral administration with onset of action occurring within 0.5-1 hour in small animals. It is distributed throughout the body with highest levels found in kidneys, plasma, erythrocytes and other tissues rich in carbonic anhydrase enzyme. It is primarily excreted unchanged in the urine. Its duration of action in small animals is about 4-6 hours.

Side effects/Adverse effects

Serious adverse effects to acetazolamide are not common, but like other sulphonamides it may cause bone-marrow depression, skin toxicity and allergic reactions. Other adverse effects which may be encountered include GI disturbances, CNS effects (sedation and depression), renal effects (crystalluria and renal colic), hypokalaemia, hyponatraemia, hyperuricosaemia and metabolic acidosis. Renal calculus formation and ureteric colic may occur due to alkaline urine leading to precipitation of calcium and phosphate crystals.

Contraindications and precautions

Acetazolamide is contraindicated in patients with significant hepatic disease, renal insufficiency, fluid and electrolyte imbalance, and hyperchloraemic acidosis. It should be used with caution in patients with severe respiratory acidosis and pre-existing haematological abnormalities.

Drug interactions

Because acetazolamide alkalises the urine, it may alter excretion rate of many drugs (e.g. quinidine and phenobarbital sodium).

Clinical uses

Acetazolamide is a week diuretic and have self-limiting action, so other diuretics have largely replaced it. In veterinary medicine, it is used for decreasing aqueous humour production in the treatment of glaucoma. Occasionally, it has also been used for its diuretic effect and in the treatment of metabolic acidosis. In human medicine, it is also used as adjunctive therapy for epilepsy and in the prophylaxis of acute high-altitude sickness.

Dose

Dogs	:	10 mg/kg, PO, 3 to 4 times daily.
Cats	:	50 mg (total), IV once.
	:	7 mg/kg, PO, 3 times daily.
Cattle, sheep & swine	:	6 – 8 mg/kg, IV, IM or SC.

Dichlorphenamide

Dichlorphenamide (Diclofenamide) is a carbonic anhydrase inhibitor that has properties and actions like acetazolamide. It has long duration of action (6-12 hours) in smaller animals and is used mainly for the treatment of glaucoma.

Dose

Dogs	:	2.5 mg/kg, PO, 2 to 3 times daily.
Cats	:	1 mg/kg, PO, 2 to 3 times daily.

Other drugs

Ethoxzolamide and methazolamide are two other carbonic anhydrase inhibitors having action similar to acetazolamide. Both drugs are sulphonamide derivative and are used mainly as diuretics and in the treatment of glaucoma. Ethoxzolamide is also used in the treatment of duodenal ulcers and some forms of epilepsy.

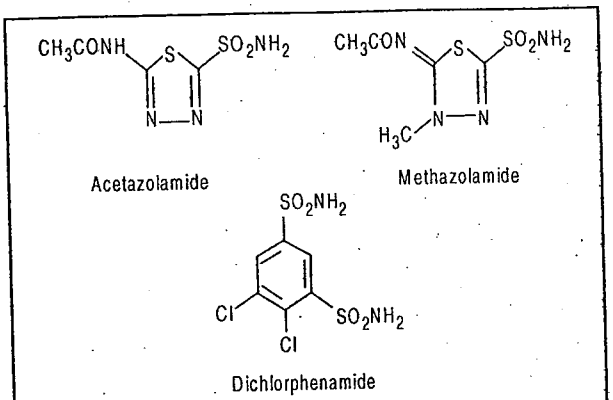


Fig. 39.3. Chemical structures of selected carbonic anhydrase inhibitor diuretics.

3. Potassium Sparing Diuretics

The potassium sparing diuretics interfere with sodium reabsorption at the distal segments of nephron and promote sodium excretion while potassium is conserved. The members of this group may act either by direct antagonism of aldosterone receptors (aldosterone receptor antagonists) or inhibition of Na^+ channels in the distal collecting tubule (inhibitors of renal epithelial Na^+ channels). As only a small fraction of Na^+ is reabsorbed at the distal tubular exchange sites, these diuretics are mild diuretics and generally used in conjunction with loop or thiazide diuretics. Such a combined therapy reduces potassium loss, minimises alkalosis, and is sometimes effective in refractory oedema.

a. Inhibitors of Renal Epithelial Na^+ Channels

Triamterene, amiloride and benzamil are the only three drugs available, which inhibit the renal epithelial Na^+ channels in the distal collecting tubule and thereby reduce the net driving force for K^+ secretion. The action of these drugs is independent of the plasma levels of aldosterone. They are mild diuretics and have no carbonic anhydrase inhibitory action.

Mechanism of action

The principal cells in the luminal membrane of late distal tubule and collecting ducts have a distinct Na^+ channels through which Na^+ enters the cell down its electrochemical gradient that is generated by Na^+-K^+-ATPase operating at the basolateral membrane (Figure 38.6). Amiloride and triamterene block these channels making less sodium available for transfer across the basolateral membrane, which indirectly inhibit K^+ excretion by abolishing the driving force for K^+ secretion. Due to the potassium-sparing capacities, hyperkalaemia (high blood potassium levels) are occasionally observed in patients taking these drugs.

Triamterene

Triamterene is a non-steroidal potassium sparing diuretic that acts directly on the distal nephron to block ion transport across the luminal face of tubular cells. Therefore, it is effective when there is no aldosterone excess.

Pharmacological effects

Triamterene blocks Na^+ channels in the late distal tubule and collecting duct and results in only a mild increase in the excretion rate of Na^+ and Cl^- (~ 2% of filtered load). Triamterene decreases H^+ ion secretion in the intercalated cells by reducing the lumen negative potential. Overall, there is enhanced excretion of Na^+, Cl^- and HCO_3^- and decreased excretion rate of K^+, H^+, Ca^{++} and Mg^{++}. Volume contraction may increase reabsorption of uric acid in the proximal tubule hence chronic administration of triamterene may decrease uric acid excretion. Triamterene has little or no effect on renal haemodynamics and does not alter the total glomerular filtration rate.

Pharmacokinetics

The Pharmacokinetics of triamterene has not been extensively evaluated in domestic animals. In humans, triamterene is incompletely absorbed orally and partly bound to plasma proteins. It is extensively metabolised in liver to an active metabolite 4-hydroxytriamterene sulphate, which is excreted in the urine.

Side effects/Adverse effects

Adverse effects with triamterene are not common and include nausea, vomiting, diarrhoea, headache, dizziness, fatigue, dry mouth and muscular cramps in humans. Serious side effects may include heart palpitations, tingling/numbness, fever, chills, sore throat, rash and back pain. It may cause hyperkalaemia and increase blood urea nitrogen and serum uric acid levels. Triamterene can also cause kidney stones through direct crystallization or by seeding calcium oxalate stones.

Contraindications and precautions

Triamterene is contraindicated in patients at risk of developing hyperkalaemia like renal failure, diabetes mellitus, metabolic acidosis, or patients taking K^+ supplements. Triamterene may impart a blue fluorescent colour to the urine.

Drug interactions

Triamterene when given with indometacin may result in acute renal failure. Combination with spironolactone and other K^+ sparing diuretics should be avoided as they may exacerbate the hyperkalaemia. Like other potassium sparing diuretics, triamterene should be avoided in combination with beta-adrenoceptor blockers which impair cellular uptake of potassium or ACE inhibitors which may pre-dispose to hyperkalaemia.

Clinical uses

Triamterene is used primarily in human medicine for the treatment of hypertension in combination with other diuretic agents, as this combination augments excretion of Na^+ while diminishing excretion of K^+. It can occasionally be used in animals to enhance the therapeutic effect of potent diuretics, especially in resistant oedema.

Dose

Dogs : 1 – 2 mg/kg, PO, 2 times daily.

Amiloride

Amiloride is an organic base and has renal actions like triamterene. It is comparatively more potent than triamterene. Similar to triamterene, amiloride works by directly blocking the epithelial sodium channel thereby inhibiting sodium reabsorption in the late distal convoluted tubules, connecting tubules and collecting ducts in the kidneys. At higher doses it also inhibits Na^+ reabsorption in proximal tubule but this is clinically insignificant. Unlike triamterene, it is not metabolised and is eliminated primarily as an intact drug in the urine. Its plasma half-life and duration of action are longer than triamterene.

Dose

Dogs & cats: 1 – 2 mg/kg, PO, once daily.

Benzamil

Benzamil (Benzyl amiloride) is a benzyl group-containing analogue of amiloride. It is similar in action to the amiloride, but its potency is several times more than the parent drug. Similar to amiloride it promotes the loss of sodium and water from the body, but without depleting potassium.

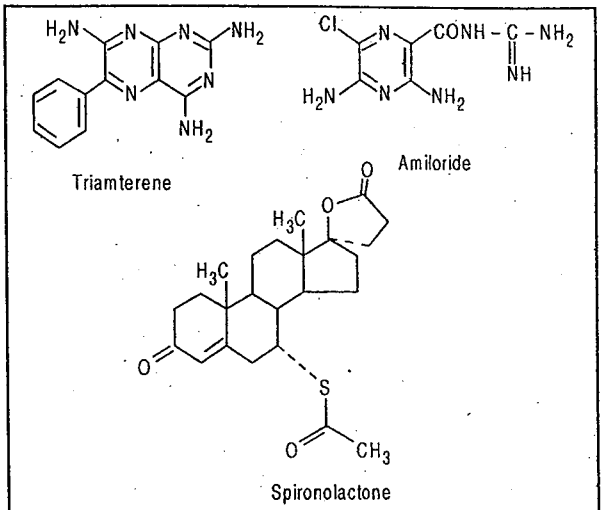


Fig. 39.4. Chemical structures of selected potassium-sparing diuretics.

b. Aldosterone Receptor Antagonists

Aldosterone receptor antagonists are a group of diuretics which act by diminishing operation of the Na^+ active reabsorption system normally activated by the endogenous steroid hormone aldosterone in the distal tubule. Although only the last few per cent of filtered sodium is subject to its control, the aldosterone is still vitally important to sodium and water conservation. Aldosterone antagonists by binding to specific mineralocorticoid (aldosterone) receptors enhance excretion of Na^+ and water, but retain K^+.

Mechanism of action

Epithelial cells in the late distal tubule and collecting duct contain cytoplasmic mineralocorticoid receptors which have high affinity for aldosterone. Normally, aldosterone secreted from adrenal cortex enters the cell from basolateral membrane and interacts with mineralocorticoid receptors to form a complex. This complex translocates to the nucleus and promotes gene-mediated mRNA synthesis that in turn directs synthesis of aldosterone induced proteins (AIPs). The AIPs activate dormant Na^+ channels and Na^+-K^+-ATPase (sodium pump), translocate Na^+ channels from cytosolic site to luminal membrane and Na^+-K^+-ATPase from cytosolic to basolateral membrane, increase expression of Na^+ channels and Na^+-K^+-ATPase, change permeability of tight junctions and increase activity of enzymes in mitochondria those are involved in ATP production (Figure 39.5). All these changes promote reabsorption of Na^+ and excretion of K^+ and H^+ (indirectly). Aldosterone antagonists such as spironolactone competitively bind to mineralocorticoid receptors, prevent synthesise of AIPs and inhibit Na^+ reabsorption. This indirectly prevents K^+ and H^+ excretion in the urine.

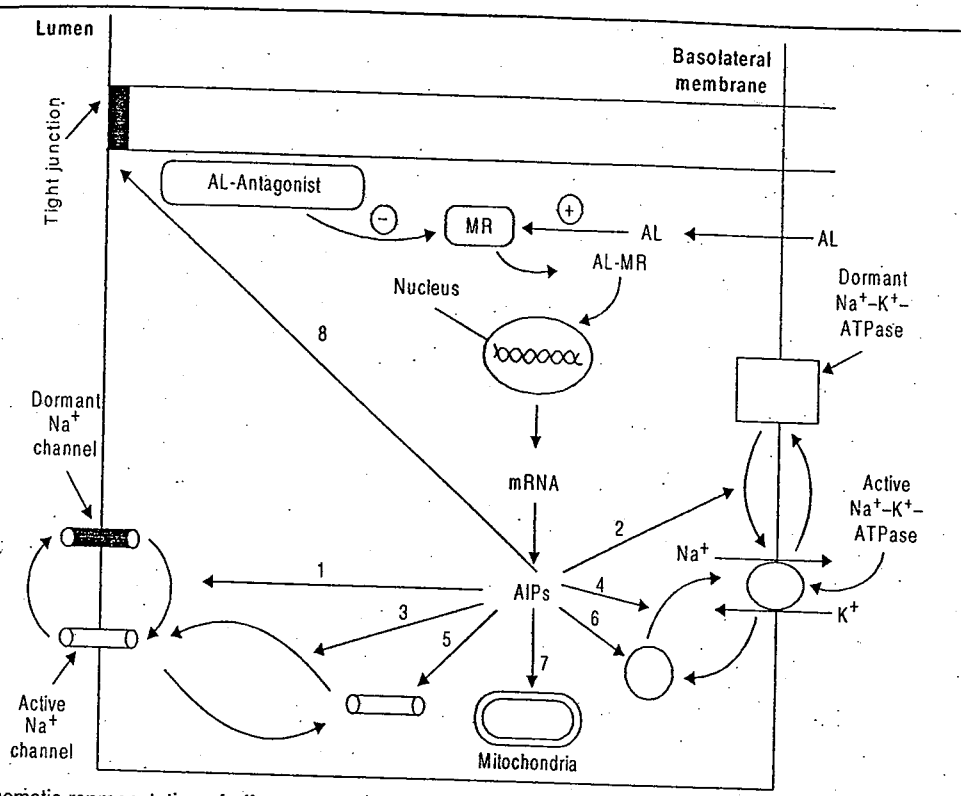


Fig. 39.5. Schematic representation of effects of aldosterone and diuretic mechanism of aldosterone antagonists.
1. Activation of dormant Na^+ channels.
2. Activation of dormant Na^+-K^+-ATPase (Sodium pump).
3. Translocation of Na^+ channels from cytoplasm to luminal membrane.
4. Translocation of Na^+-K^+-ATPase from cytoplasm to basolateral membrane.
5. *De novo* synthesis (expression) of Na^+ channels.
6. *De novo* synthesis (expression) of Na^+-K^+-ATPase.
7. Increased mitochondrial production of ATP.
8. Changes in permeability to tight junction.

Abbreviation : AIPs = aldosterone - induced proteins ; AL = aldosterone ; MR = mineralocorticoid receptors ; AL-MR = aldosterone – mineralocorticoid receptor complex ; m-RNA = messenger ribonucleic acid. + = activation ; – = inhibition.

Aldosterone receptor antagonists have a very slow onset of action, taking several days to develop. Similarly the effect of these drugs also diminishes slowly. This is because steroid pathways alter gene transcription and it takes several days for the gene products to change.

Spironolactone

Spironolactone is a synthetically produced steroid lactone that is chemically related to the mineralocorticoid aldosterone (Figure 39.4). It acts by competing with aldosterone for the site on mitochondrial receptors in renal tubule cells with consequent loss of the sodium conserving gene products.

Pharmacological effects

Spironolactone is only a weak diuretic because its effects target the distal nephron (collecting tubule), where urine volume can only be slightly modified. However, it efficiently antagonises K^+ loss induced by other diuretics and slightly adds to the natriuretic effect. The effect of spironolactone on urinary excretion is very similar to those induced by renal epithelial Na^+ channel inhibitors (e.g. triamterene), but unlike these drugs the clinical efficacy of spironolactone is dependent on the endogenous levels of aldosterone. It is observed that the pharmacological effect of spironolactone largely depends on the activity of aldosterone i.e. greater the effect of aldosterone on urine composition, the greater is the effect of spironolactone on urinary excretion.

In general, spironolactone causes increased excretion of Na^+, Cl^- and water and decreased excretion of K^+, NH_4^+, phosphate and H^+. It has no effect on carbonic anhydrase inhibition or renal transport mechanisms. It has little or no effect on renal haemodynamics and does not alter total glomerular filtrate.

Higher concentrations of spironolactone have been reported to interfere with steroid biosynthesis, but this effect has limited clinical relevance. Spironolactone has anti-androgen activity by binding to the androgen receptor and preventing it from interacting with dihydrotestosterone.

Pharmacokinetics

Information on pharmacokinetics of spironolactone is limited in domestic animals. In humans, spironolactone is well absorbed (>90%) after oral administration. It is highly bound to plasma proteins, undergoes enterohepatic cycle and is completely metabolised in liver. One of its metabolite canrenone is active. The half-life of spironolactone is short (1-2 hours) but that of active metabolite canrenone is long (16.5 hours), which prolongs the biological effects of spironolactone. The diuretic effect of spironolactone (when used alone) is gradually attained and reaches its maximal effect on the third day of therapy. It is the only diuretic

that does not require access to the tubular lumen to induce a diuresis.

Side effects/Adverse effects

Adverse effects of spironolactone are usually mild and may include electrolyte (hyperkalaemia and hyponatraemia) and water imbalance. Gastrointestinal distress (vomiting, diarrhoea, bleeding and peptic ulcers), CNS effects (lethargy, ataxia and drowsiness), skin rashes and rarely blood dyscrasias may also occur. Due to its steroid structure, spironolactone affects androgen and other steroid receptors and may cause gynaecomastia, impotence, testicular atrophy and other endocrine changes in humans. Overdosage or prolonged use of spironolactone can cause serious hyperkalaemia.

Contraindications and precautions

Spironolactone is contraindicated in patients with hyperkalaemia, anuria, acute renal failure, or metabolic acidosis and diabetes mellitus. It should be used cautiously in any renal impairment or hepatic disease.

Drug interactions

Spironolactone should not be used concurrently with other potassium-sparing diuretics as hyperkalaemia may occur. It should be avoided concurrently with ACE inhibitors and indometacin, which may enhance risk of hyperkalaemia. Potassium supplements and salt substitutes containing potassium should not be administered during spironolactone therapy.

Clinical uses

The use of spironolactone in domestic animals is limited. It is often used in combination with other diuretics (e.g thiazides or loop diuretics) in prevention of hypokalaemia occurring during diuresis. Spironolactone is particularly useful in the treatment of primary hyperaldosteronism (e.g. adrenal adrenomas) and in the treatment of refractory oedema associated with secondary aldosteronism (cardiac failure, hepatic cirrhosis, nephritic syndrome and severe ascites). Due to its antiandrogenic effect, spironolactone is occasionally used for treating hair loss and acne in women, and can be used as a topical medication for treatment of male baldness.

Dose

As a diuretic in CHF and ascites

Dogs & cats: 2 – 4 mg/kg, PO, once daily.

Eplerenone

Eplerenone is another aldosterone antagonist, with mechanism of action and pharmacological effects similar to those of spironolactone. However, it is considered to be more specific for the mineralocorticoid receptor and is used mainly in adjunct therapy for reducing cardiovascular risk in patients following myocardial infarction. Common adverse drug effects as observed in human patients include hyperkalaemia, hypotension, dizziness, altered renal function and increased creatinine concentration.

Other drugs

Canrenone and potassium canrenoate are other aldosterone antagonists which are available for use in human patients. Canrenone is basically an active metabolite of spironolactone that has been synthesised as a drug and has properties and action like spironolactone. Canrenoate is a pro-drug that is not active *per se*, but is converted to canrenone in body.

IV. MISCELLANEOUS DIURETICS

1. Acidifying or Alkalinising Salts/Saline Diuretics

a. Acidifying Salts/Urinary Acidifiers

Some salts used primarily for acidification of urine also provide a diuretic effect and are known as saline diuretics. These agents are now rarely used clinically for their diuretic effect.

Table 39.1: Urinary electrolyte pattern, acid-base balance and efficacy of some important diuretics.

	Diuretic	Main site of action in nephron	Mechanism of action	Efficacy	Urinary electrolyte excretion					Max. % of filtered Na^+ excreted	Acid-base imbalance
					Na^+	K^+	H^+	Cl^-	HCO_3^-		
1.	Furosemide	Medullary thick ascending limb of loop of Henle	Inhibition of Na^+-K^+-$2Cl^-$ symporter	High	+++	+	+	++	+	25	Alkalosis
2.	Etacrynic acid	Medullary thick ascending limb of loop of Henle	Inhibition of Na^+-K^+-$2Cl^-$ symporter	High	+++	+	+	+++	–	25	Alkalosis
3.	Chlorothiazide	Distal convoluted tubules	Na^+-Cl^- symporter	Moderate	++	+	+	+	+	10	Alkalosis
4.	Mannitol	Proximal tubule, descending limb of loop of Henle	Induction of osmotic diuresis	Low to moderate	++	+		+	+	10	
5.	Acetazolamide	Proximal tubule	Inhibition of carbonic anhydrase enzyme	Low	+	++	–	+/–	++	5	Acidosis
6.	Triamterene	Distal collecting tubule	Inhibition of epithelial sodium channels	Low	+	–	–	+	0	3	Acidosis
7.	Spironolactone	Distal collecting tubule	Inhibition of aldosterone receptors	Low	+	–	–	+	0	3	Acidosis

+ = Increase; – = decrease; +/– = may increase or decrease; 0 = no change.

Ammonium chloride

Ammonium chloride is an acidifying salt that possesses some diuretic effect. However, it is rarely used as a diuretic presently because it has a limited and short-term effect. Development of metabolic acidosis is an important adverse effect of ammonium chloride. The pharmacology of ammonium chloride is described in Chapter 41.

Sodium chloride

Sodium chloride (common salt) is used in the control of urolithiasis in veterinary medicine. Inclusion of sodium chloride in the ration along with readily available drinking water assures adequate water intake and thereby adequate flushing of the urethra. Additionally, sodium chloride increases the surplus ion (Na^+) content effect due to increased Na^+ excretion in the urine.

b. Alkalinising Salts

The potassium salts including potassium citrate and potassium acetate have been used in the past as diuretics, but they are potentially dangerous and their use is discouraged. They tend to alkalinise urine and increase its volume slightly. Presently, they are mainly used to change the pH of urine, rather than as diuretics.

2. Methylxanthines/Xanthine Derivatives

The methylxanthines have long been known for their diuretic action. These drugs are weak diuretics which act by increasing the renal blood flow both by cardiac stimulation (increased cardiac output) and by better renal haemodynamics causing an increase in glomerular filtration rate. In addition, they also act directly by inhibiting reabsorption of Na^+ and water in the proximal convoluted tubule. However, there is no change in acid-base balance or K^+. loss. The methylxanthines as diuretics have a limited use as they possess mild action and on repeated use the response is lost. Sometimes, aminophylline (theophylline ethylnediamine) is used in the management of acute pulmonary oedema, but its efficacy is mainly due to its cardiac and bronchial action rather than its diuretic action (see for detail Chapters 32 and 46).

V. NEWER DRUGS

1. Aquaretics

Aquaretics are a group of pharmaceuticals which promote excretion of water without electrolyte loss. They are not strictly speaking diuretics, but are sometimes classified as such. These drugs are used primarily in the treatment of hyponatraemia.

a. Vaptans/Vasopressin Receptor Antagonists

Vaptans are a new class of drugs which antagonize the action of vasopressin, also known as anti-diuretic hormone (ADH), on various vasopressin receptor subtypes. These drugs are considered useful in the treatment of water overload or hyponatremia. Many of these drugs are already in clinical use (e.g. conivaptan, mozavaptan and tolvaptan) or in clinical trials (e.g. lixivaptan and satavaptan).

Mechanism of action

Vaptan drugs act by inhibiting the action of vasopressin on its receptors (V_{1A}, V_{1B} and V_2). These receptors have a variety of peripheral and central functions (Chapter 49). The V_{1A} receptors are expressed both peripherally and centrally and are involved mainly in modulation of blood pressure (peripheral action) and behavioural functions (central action). The V_{1B} subtype is located

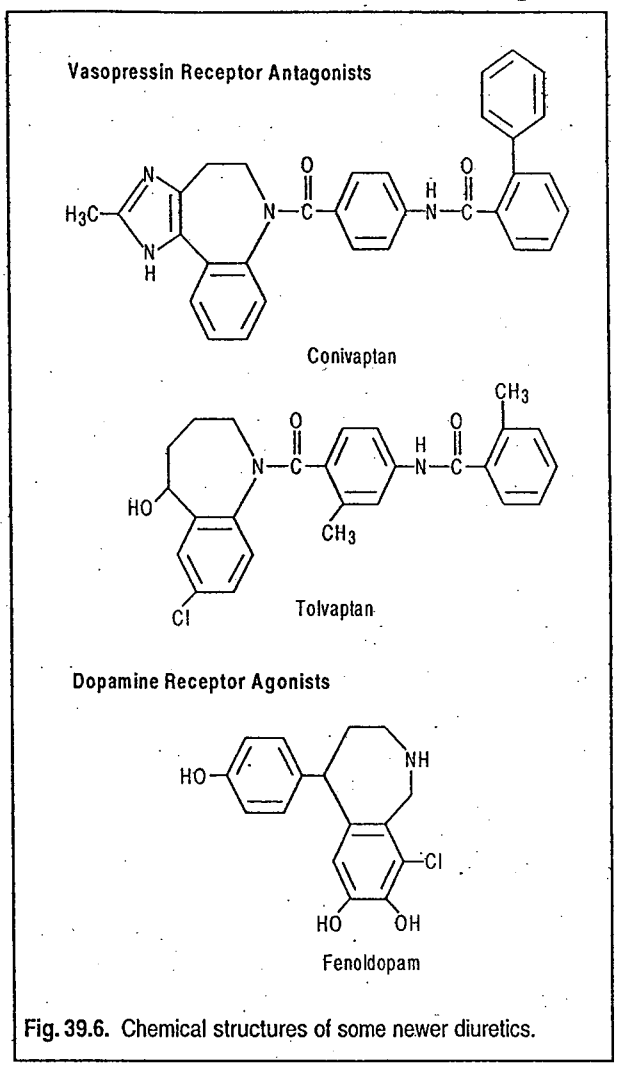


Fig. 39.6. Chemical structures of some newer diuretics.

in the adenohypophysis, whereas the V_2 receptors are expressed only peripherally. The V_2 receptors are located mainly in the principal cells of renal collecting duct system and are involved in the modulation kidney function. The affinity of vaptans for various vasopressin receptor subtypes is variable and accordingly their pharmacological effects are also variable.

Vaptans blocking V_2 vasopressin receptors in kidneys prevent the ADH hormone from stimulating the appearance of water channels, known as aquaporins, in the collecting duct. In the absence of aquaporins, the collecting duct becomes impermeable to water as a result of which water is not absorbed in the terminal segment of nephron. Appearance of more water in urine without the loss of mineral salts, which often accompanies other diuretics, induces increased urine volume.

Conivaptan

Conivaptan is a non-peptide vasopressin (antidiuretic hormone) receptor antagonist. It is a non-selective vaptan drug with action on two of the three subtypes of the vasopressin receptor (V_{1A} and V_2). Conivaptan is used primarily in treatment of euvolaemic hyponatraemia, a condition in which the salt balance is normal but there is too much water (water overload). This condition is most often seen in the syndrome of inappropriate antidiuretic hormone (SIADH). Current treatments for this condition are not pharmacological and are often not satisfactory. Conivaptan could also be used to manage hypovolaemic hyponatraemia, a

condition in which patients have both too much salt and too much water in their bodies. In addition to hyponatraemia, conivaptan has been tried for the treatment of decompensated congestive heart failure and has been found effective.

Important side/adverse effects associated with conivaptan in human patients include vomiting, increased thirst, headache, hypokalaemia, peripheral oedema, diarrhoea, polyurea and pain at injection site. Close monitoring of serum sodium levels is required during treatment, as overly rapid increases in serum sodium levels have been observed in some cases.

Tolvaptan

Tolvaptan is another vasopressin receptor antagonist with pharmacological effects similar to those of conivaptan. However, unlike conivaptan, it is a selective vasopressin V_2-receptor blocker. It is used orally and works by producing aquaresis by blocking the effects of vasopressin. It is well absorbed after oral administration with peak effect occurring in about 2-3 hours. Drug is metabolized mainly in liver by cytochrome P-450 system. It is approved for treatment of hyponatraemia associated with congestive heart failure, cirrhosis and syndrome of inappropriate antidiuretic hormone (SIADH) due to ADH producing tumours. The most frequently reported adverse events are thirst and dry mouth, which result from the pharmacodynamic effects of the drug.

2. Dopamine Receptor Agonists

Fenoldopam

Fenoldopam is a selective dopamine D_1 receptor weak partial agonist. It is suggested that stimulation of peripheral D_1 dopamine receptors in renal vasculature enhances blood supply to kidney that in turn increases glomerular filtration rate and induces diuresis and natriuresis. Fenoldopam also has significant vasodilatory effects on other peripheral blood vessels including the mesenteric and coronary vasculature, which produces hypotension. Fenoldopam is used mainly in human medicine as an antihypertensive drug. In dogs and cats, it has been investigated as diuretic and found useful. The drug is formulated as a solution to be diluted for intravenous infusion.

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CHAPTER 40 DRUGS ACTING ON FLUID, ELECTROLYTE AND ACID-BASE BALANCE

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PHYSIOLOGY

Water Balance

Next to oxygen, water is most important for life. Water and solutes (electrolytes and non-ionised organic molecules) constitute the body fluids, which are essentially required for various physiological processes and maintenance of homeostasis. Water is also central to acid-base neutrality and enzyme function. The volume and composition of body fluids vary within a narrow range from day to day because there is continuous exchange of water and solutes with the external environment as well as with the different compartments of body. Under normal steady state conditions, the fluid intake is carefully matched by equal output from the body to prevent body fluid volumes from increasing or decreasing.

Approximately 60% of a normal animal's body weight is composed of water, with variations between and within species. Obese and older animals tend to have a smaller percentage of water, whereas neonates can have as much as 80% of their body weight as water. Of the 60% water, approximately two-third (40%) is within the cells called intracellular fluid (ICF) and remaining one-third (20%) is outside the cells referred to as extracellular fluid (ECF). The ECF may be further divided into intravascular fluid (blood plasma), extravascular fluid (interstitial fluid, lymph), inaccessible bone fluid (water trapped in deep layers of bone, not readily exchangeable) and transcellular fluid (secretions of glandular tissues, gastrointestinal fluid, respiratory fluid, aqueous humour, peritoneal fluid, cerebrospinal fluid, etc.). Although the ECF and ICF differ markedly in electrolyte composition, they are in osmotic equilibrium and water is freely diffusible between them. The relative volume distribution of water between these compartments (ECF and ICF) is largely governed by the number of osmotically active particles in each compartment. As the ECF is an all-pervading fluid surrounding the cells, its composition is kept constant within narrow limits, as regards to its osmolality, chemical and ionic composition, and pH.

Electrolyte Balance

Electrolytes are compounds which exist as charged particles in aqueous solution and conduct a current in electricity. The positively charged particles are cations and the negatively charged particles are anions. Electrolyte solutions are normally formed when a salt is placed into a solvent such as water and the individual components dissociate due to the thermodynamic interactions between solvent and solute molecules. An electrolyte in a solution may be described as concentrated if it has a high concentration of ions, or dilute if it has a low concentration of ions. If a high proportion of the solute dissociates to form free ions, the electrolyte is strong; if most of the solute does not dissociate, the electrolyte is weak.

The ECF contains mainly Na^+ as cation accompanied by anions like Cl^-, HCO_3^- and HPO_4^{2-}, while the ICF contains mainly

K^+ as cation accompanied by a wide variety of anions such as HCO_3^-, PO_4^{3-}, SO_4^{2-} and citrate. These electrolytes dissolved in body fluids fulfil vital roles in virtually all of life processes like nerve conduction, muscle contraction and metabolic reactions. The movement of cations, mainly Na^+ and K^+, play a major role in the functional activity of cells, and they also maintain their osmotic pressure and size of fluid compartments of the body. The ECF volume is determined mainly by the ECF sodium content and ICF volume is a function of ICF potassium content. The extracellular preponderance of sodium ions and the intracellular preponderance of potassium ions are maintained by membrane bound Na^+-K^+-ATPase pump that exchanges sodium and potassium ions. Concentrations of some important electrolytes in extracellular and intracellular fluids are given in Table 40.1.

Table 40.1: Concentration of important electrolytes in extracellular and intracellular fluids.

Electrolyte	Extracellular Fluid (mEq/L)	Intracellular Fluid (mEq/L)
Cations		
Sodium (Na^+)	142	10
Potassium (K^+)	4.5	150
Calcium (Ca^{++})	2.5	-
Magnesium (Mg^{++})	1.0	40
Total	**150**	**200**
Anions		
Chloride (Cl^-)	104	-
Bicarbonate (HCO_3^-)	24	10
Phosphate ($H_2PO_4^-$, HPO_4^{-2})	2	140
Protein	14	40
Others (sulphate, lactate)	6	10
Total	**150**	**200**

Cited values are approximate and represent general levels for all species.

Acid-Base Balance

The balance between acidity and alkalinity in animal body is referred to as acid-base balance. The acid-base balance is an important homeostatic mechanism and is determined mainly by the hydrogen ion concentration in various body fluids. The hydrogen ion concentrations (pH) of extracellular fluid is maintained within remarkably narrow limits at about 40 nEq/L (pH 7.4) in arterial blood and about 45 nEq (pH 7.35) in venous blood and interstitial fluid. Even at these low concentrations, hydrogen ions have profound effects on metabolic events largely through interaction with cellular proteins. As most enzymatic reactions have a narrowly defined range of pH optimum, a small variation in the hydrogen ion concentrations have direct effects on the rates of reactions and thus many biological processes. In general, pH of ECF below 7.0 or above 7.8 is incompatible with life, although animals can live for a few hours at a pH of 6.8 or 8.0. Normally a pH value of arterial blood below 7.4 is classified as acidosis or acidaemia and a pH value of above 7.4 is referred to as alkalosis or alkalaemia.

The hydrogen ion concentration or the pH is tightly regulated within an acceptable range by several buffering or defence mechanisms. These defence mechanisms include chemical buffer systems, respiration and renal excretions of acid and base.

1. Chemical buffer systems

The chemical buffer systems of body are able to take up or release hydrogen ion so that changes in hydrogen ion concentration (pH) are minimised. The buffer system consists of a weakly dissociated acid and its sodium or potassium salt. A buffer system can be effective in keeping the pH of the medium unchanged only as long as both components of the buffer system are available. The general form of the buffering reaction is:

$$\text{Buffer} + H^+ \rightleftharpoons H\ \text{Buffer}$$

In this example, a free H^+ combines with the buffer to form a weak acid (H buffer), which can either remain as an undissociated molecule or dissociate back to buffer and H^+. When the hydrogen ion concentration increases, the reaction is forced to the right and more H^+ bind to the buffer. Conversely, when the hydrogen ion concentration is low, the reaction shifts toward left and hydrogen ions are released from the buffer. The three most important buffer systems of body are the bicarbonate buffer system, the phosphate buffer system and the protein buffer system.

a. ***The bicarbonate buffer system:*** The bicarbonate buffer system or bicarbonate-carbonic acid (HCO_3^-/H_2CO_3) buffer system consists of a weak acid, the carbonic acid (H_2CO_3), and a bicarbonate salt ($NaHCO_3$). Carbonic acid, formed by the reaction of CO_2 with H_2O, ionises weakly to form small amounts of H^+ and HCO_3^-, while HCO_3^- combines with excess H^+ to form H_2CO_3. At pH 7.4, the normal ratio of HCO_3^- : H_2CO_3 is 20: 1 in blood.

$$CO_2 + H_2O \underset{\text{Carbonic anhydrase}}{\rightleftharpoons} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

When the H^+ concentration decreases (or OH^- concentration increases) in plasma, more of H^+ and HCO_3^- are formed from carbonic acid. The concentration of H_2CO_3 decreases, thereby causing more of CO_2 to combine H_2O to replace the H_2CO_3. The decreased CO_2 inhibits respiration and reduces rate of CO_2 expiration. The rise in blood HCO_3^- that occurs is compensated by increased renal excretion of HCO_3^- (The blood HCO_3^- concentration is normally monitored by the kidney). The opposite reaction takes place when there is excess of H^+ in plasma. The excess of H^+ is buffered immediately by HCO_3^- to form H_2CO_3 and then CO_2 and water. The excess CO_2 stimulates respiration, which eliminates the CO_2 from ECF.

The carbonic acid-bicarbonate system is not a very powerful buffer system, but its plentiful supply and backing by physiological mechanisms makes it the most important extracellular buffer in the body. As discussed above, lungs and kidneys are important regulatory organs of the bicarbonate carbonic acid buffer system.

b. ***The phosphate buffer system:*** The main elements of phosphate ($HCO_4^{-2}/H_2PO_4^-$) buffer system are $H_2PO_4^-$ (NaH_2PO_4), a weak acid, and HPO_4^{-2} (Na_2HPO_4), a weak base. Like the bicarbonate-carbonic acid system, this system can take up either H^+ or release H^+.

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{-2}$$

The phosphate buffer system is not of major importance as an extracellular fluid buffer, but plays a major role in buffering renal tubular fluid and intracellular fluids.

c. ***Protein buffer system:*** The proteins of cells and plasma are most abundant buffers of the body because of their high concentrations, especially within the cells. Proteins are formed from amino acids, whose free acidic (-COOH) or basic ($-NH_2$) group can react with bases or acids, respectively. The protein buffers inside the cells, therefore, act as a first line defence against gross changes of pH. Other intracellular buffers include organic and inorganic phosphates, and haemoglobin in the red cell. All these buffer systems inside the cell help to regulate the pH of ECF because elements of bicarbonate buffer system (e.g. CO_2, H^+ and also HCO_3^-) can also diffuse to variable extent inside the cell.

2. Respiratory regulation of acid-base balance

The lungs control acid-base balance by controlling the CO_2 concentration in ECF. As discussed above, an increase in ventilation eliminates CO_2 from ECF, which by mass action reduces the hydrogen ion concentration. Conversely, a decreased ventilation increases CO_2, thus increasing hydrogen ion concentration in the ECF. In general, the respiratory regulation of acid-base balance is rapid and is about one to two times as great as buffering power of all other chemical buffers in the EFC.

3. Renal control of acid-base balance

The renal control of acid-base balance is a slow but an important process. The kidneys control acid-base balance by excreting either acidic or basic urine, thereby reducing the amount of acid or base in the ECF, respectively. When there is a reduction in the H^+ concentration in ECF (alkalosis), kidneys enhance excretion of HCO_3^- by not reabsorbing all the filtered bicarbonate. This helps to raise the ECF hydrogen ion concentration back towards normal. On the other hand, in excess of H^+ (acidosis), kidneys reabsorb all the filtered HCO_3^- and also produce new HCO_3^-. In addition, excess H^+ is exchanged for Na^+ in the renal tubules, so that H^+ is excreted and Na^+ is reabsorbed. In this way, H^+ (acidity) is excreted and Na^+ and HCO_3^- (alkalinity) is retained, thereby decreasing the ECF hydrogen ion concentration back towards normal.

DISTURBANCE OF WATER, ELECTROLYTE AND ACID-BASE BALANCE

Disturbance in water, electrolytes and acid-base balance involves four major properties of body fluids – Volume (water), osmolality, hydrogen ion concentrations (pH), and specific ion concentration. These disturbances may be seen either in one or in all properties of fluid.

WATER/VOLUME IMBALANCE

Water deficit/Dehydration

Water deficit or Dehydration (Hypohydration) is a condition in which there is excessive loss of body water, usually accompanied by the disturbances of electrolyte metabolism, resulting in hypovolaemia. Decrease in volume may be life threatening because it results in decrease in circulating blood volume, decrease in cardiac output, and also decrease in microcirculation. Dehydration results from inadequate fluid intake in the face of normal or increased fluid losses. Dehydration is common in domestic animals. Clinical signs of dehydration are said to be first apparent with fluid losses equivalent to 4-6% of body water. Moderate dehydration is said to be present with fluid losses of 8-10%, and severe dehydration when fluid losses are greater than 12% of body weight.

The clinical signs of dehydration include weight loss, altered skin turgor, sunken eyes, dry mucous membrane and reduced urine output (See Table 40.5). The clinical consequences of dehydration depend much more on the pattern of electrolyte loss than the absolute water deficit. Depending on the associated electrolyte losses, dehydration may be broadly divided into two types: hypertonic dehydration and isotonic dehydration.

a. *Hypertonic dehydration:* Hypertonic dehydration is a condition in which water losses occur with little or no electrolyte (e.g. sodium and potassium) losses. This type of imbalance between the total body water and electrolyte generally results in enhanced serum sodium concentration and ECF osmolality. Hypernatraemia is associated with contraction of ICF volume and shrinkage of the cells. Hypertonic dehydration usually results during excessive sweating, panting, hyperventilation, water restrictions, ketoacidosis, fever, hot environment and ADH deficiency. Increased water loss may be due to osmotic diuresis in diabetes mellitus.

b. *Isotonic dehydration:* Isotonic dehydration is a condition in which water losses are associated with proportionate losses of exchangeable electrolytes. With this type of isotonic fluid loss, plasma sodium concentration remains within normal limits despite the development of large sodium deficit. The animal exhibits decrease in plasma and ECF volume with clinical signs of inadequate circulating fluid volume and deficiency of sodium. Common causes of isotonic dehydration include acute diarrhoea in most species, heavy sweating in horses and inappropriate diuretic administration.

Treatment: Treatment of water deficit depends on the type of dehydration. Hypertonic dehydration (water loss in excess of electrolytes) is treated with hypotonic fluids. In hypertonic dehydration, water must be provided initially without electrolytes, and dextrose 5 percent in water (D5W) is recommended. If more fluid is needed after plasma has reached normal tonicity, 0.9 percent sodium chloride solution may be used. In case of isotonic dehydration (isotonic loss of water and sodium), isotonic saline solution (0.9 percent NaCl) or balanced isotonic electrolyte solution may be used to restore the ECF volume.

Water excess/Overhydration

Water excess or overhydration does not occur commonly in animals because over hydration is usually balanced through appropriate renal water excretion. Water excess can occur iatrogenically as a result of excessive fluid administration to patients with compromised renal function. If these fluids provide free water, as with 5% dextrose solution, plasma sodium concentration will decrease thereby resulting in hypotonicity of ECF. As the ECF and ICF share the water excess proportionately, the hypotonicity of ECF is associated with expansion of ICF and thus swelling of cells. However, if the IV fluids consist of isotonic sodium containing replacement fluids such as saline or lactated

Ringer's solution, there will be no change in plasma sodium concentration and osmolality, but there will be an increase in plasma and ECF volume with potential for cardiovascular overload, cerebral oedema, pulmonary oedema or generalised oedema formation.

Treatment: Treatment of water excess in body is done usually with diuretics.

ELECTROLYTE IMBALANCE

Sodium

Sodium is an important electrolyte required for homeostasis and a number of physiological processes. Sodium ions are essentially required in nerve and cardiac functioning, and in influencing osmotic balance between cells and the interstitial fluid. The ECF volume contains approximately one-half to two-thirds of the body sodium that amounts to essentially all of the body's readily available and exchangeable sodium. The remainder one-third to one-half of body sodium is bound to skeletal bone and is not rapidly exchangeable. As discussed earlier, the exchangeable sodium content is the principal determinant of ECF volume and also osmolality. Therefore disturbances in sodium concentration affect not only the water balance, but also other physiological processes. Normally ECF contains about 140 mEq/L of sodium, while ICF contains about 10 mEq/L of sodium (Table 40.1).

Sodium deficit/Hyponatraemia

Hyponatraemia is a condition when serum concentration is less than normal (i.e. <140 mEq/L in dogs). Under normal conditions, the kidneys regulate sodium loss from body, excreting as much sodium as ingested. Hyponatraemia develops when the patient is unable to excrete ingested water or when urinary and insensible fluid losses have a combined osmolality greater than that of ingested or parenterally administered fluids. Sodium depletion is almost invariably associated with excessive losses of sodium containing fluids and occurs rarely as a result of dietary sodium deficiency. A decrease in serum sodium concentration is usually associated with plasma hypoosmolality, although some patients with decreased serum sodium concentration may show normal plasma osmolality (e.g. in hyperlipaemia or hyperproteinaemia) or increased plasma osmolality (e.g. in hyperglycaemia or mannitol infusion). Patients with hyponatraemia and decreased plasma osmolality may be hypovolaemic (mainly), hypervolaemic or normovolaemic.

a. *Hyponatraemia with volume depletion/Hypovolaemic-hyponatraemia:* Hypovolaemic hyponatraemia is a condition when both water and sodium levels are low. A decrease of serum sodium usually associated with plasma hypovolaemia mainly occurs due losses of sodium containing fluid by renal or non-renal routes. Fluid and salt loss by the renal route may be due to hypoadrenocorticism, diuretic administration or salt losing nephropathies (e.g. chronic pyelonephritis, primary urinary tract obstruction, polycystic renal disease). Gastrointestinal (e.g. vomiting and diarrhoea), third space (e.g. peritonitis and pancreatitis) (accumulation of sodium containing fluid within body cavities is referred to as third space), and cutaneous (e.g. burns, excessive sweating) losses constitute the non-renal routes of sodium loss. Mastitis also markedly enhances sodium depletion in milk and could play a role in hyponatraemia in lactating cows maintained on a low sodium diet.

b. *Hyponatraemia with volume excess/ Hypervolaemic-hyponatraemia:* Hypervolaemic-hyponatraemia is a condition in which excess water dilutes the sodium concentration, causing low sodium levels. Hyponatraemia with volume excess is observed in clinical conditions like nephritic syndrome, congestive heart failure and severe liver disease. In these disorders, there is impaired free water excretion mainly due to stimulation of vasopressin release, which causes dilution of retained sodium.

c. *Hyponatraemia with normal volume/ Normovolaemic-hyponatraemia:* Normovolaemic-hyponatraemia (Euvolaemic hyponatraemia) is a condition in which normal water levels are combined with low sodium levels. Hyponatraemia with normal volume may occur as a result of primary (psychogenic) polydipsia, administration of hypotonic fluids and impaired free water clearance due to renal disease or drugs with antidiuretic effects.

The clinical signs of sodium deficit are observed mainly in acute hyponatraemia and are related more to the rapidity of onset than to the severity of the associated plasma hypoosmolality Reduction in plasma osmolality causes influx of water into the central nervous system. Early signs include lethargy, nausea and vomiting, whereas more severe signs include pulmonary and cerebral oedema, coma and a marked increase in body weight. Seizures may also result from acute water intoxication. Clinical signs may be absent in chronic disorders mainly due to a slow decrease in serum sodium concentration and plasma osmolality.

Treatment: Treatment of hyponatraemia includes the management of underlying cause and correction of serum concentration and plasma osmolality. Severe, symptomatic hyponatraemia of rapid onset may require prompt treatment with hypertonic saline solution (3-5% NaCl). In more common mild to moderate hyponatraemia with true volume deficit, IV infusion of isotonic saline solution (0.9% NaCl) may be sufficient. Hyponatraemia due to adrenocortical insufficiency should be treated with hormone replacement therapy. Hypertonic saline solution may be administered concurrently with loop diuretics to affect more rapid correction of hyponatraemia in overhydrated patients. In normovolaemic patients with hyponatraemia (e.g. psychogenic polydipsia), water should be carefully restricted to an amount less than urine output and drug causing antidiuretic effect should be discontinued.

Sodium excess/Hypernatraemia

Hypernatraemia is a condition when serum sodium concentration is more than normal (i.e. >155 mEq/L in dogs and >160 mEq/L in cats). Hypernatraemia can only develop if water is lost from the animal in excess of sodium (mostly) or if sodium containing salts are ingested or administered therapeutically (rarely). Hypernatraemia may be associated with an increased ECF, a normal ECF or a decreased ECF.

a. *Hypernatraemia with increased ECF volume/ Hypervolaemic hypernatraemia:* Sodium excess occurs most often in association with an increase in body water leading to an isotonic expansion of ECF volume and development of hypertension or generalized oedema. This condition mainly occurs due to excessive intake of sodium.

Accordingly excess salt in feed, saline emetics, ingestion of seawater and administration of high sodium-containing drugs are the most important causes for this condition. Hypervolaemic hypernatraemia is not much clinically important in animals because most domestic animals can tolerate a large sodium intake provided they have adequate drinking water. Salt intoxication occurs in animals only when they are deprived of fresh water. In humans, hypernatraemia is reported with mineralocorticoid excess.

b. *Hypernatraemia with decreased ECF volume/ Hypovolaemic hypernatraemia:* Hypovolaemic-hypernatraemia occurs in animals typically due to losses of hypotonic fluid and their inability to replace sufficient water to normalise their serum osmolality. Hypovolaemic-hypernatraemia occurs in a number of conditions in veterinary medicine and is the most frequent situation clinically. It is commonly seen in diarrhoea, vomiting, hypoadrenocorticism, diabetes mellitus, acute and chronic renal failure, profuse sweating, severe burns, fever, IV solute administration, osmotic and lop diuretics, etc. Inadequate intake of water, typically in disabled patients which are unable to drink water is also a common cause of hypernatraemia.

c. *Hypernatraemia with normal ECF volume/ Isovolaemic-hypernatraemia:* Isovolaemic hypernatraemia (Euvolaemic hypernatraemia) occurs in animals those have essentially pure water losses i.e. water loss is not accompanied by significant electrolyte loss. It mainly occurs in animals with pituitary diabetes insipidus, febrile illness, hot and dry climates and thyrotoxicosis. Generally in such conditions, animal ingests sufficient water to maintain serum osmolality within normal limits. Life threatening problems may arise only if animal has limited access to water or in conditions such as CNS depression when animal cannot drink normally.

Clinical signs associated with hypernatraemia are related mainly to the cerebral dehydration. These include depression, lethargy, muscle rigidity, tremors, myoclonus and hyper-reflexia. Other signs include vomiting, diarrhoea, polyuria and polydipsia. Seizures, coma and death may occur in more severe cases. The severity of clinical conditions is related directly to the rate of onset of hypernatraemia and concentration of sodium.

Treatment: Specific treatment of hypernatraemia depends on the type and extent of hypernatraemia. In hypernatraemia associated with pure water loss, the elevated sodium may be lowered by providing water either orally or in form of 5% dextrose in water (D5W) by IV route. Other isotonic fluids may also be considered. In general, not more than 50% of the calculated water deficit is replaced in first 24 hours; the remaining deficit is replaced over next 2-3 days. Specific problems such as pituitary diabetes insipidus, fever, polypnoea and high ambient temperature which are causing hypernatraemia should also be treated appropriately. In hypernatraemia associated with hypotonic fluid loss, 0.45% sodium chloride solution or other hypotonic fluids may be used to replace water. In a hypernatraemic patient, use of 0.9% normal saline may also be considered because isotonic saline is hypotonic relative to the patient's plasma and thus a dilution effect may occur.

In salt poisoning (hypernatraemia associated with volume expansion), the saline elimination should be augmented through use of loop diuretics, if the renal function is normal. Intravenous D5W is also used to slowly reduce the hypernatraemia. Such patients, however, should not be treated with hypotonic fluids or allowed to take excess water because such patients already have increased blood volume, and such treatment will aggravate oedematous conditions (e.g. pulmonary oedema).

Potassium

Potassium is the most important cation found inside the cell with more than 98% of exchangeable potassium located intracellularly. Normally, about 140 mEq/L concentration of potassium is inside the cell and only about 4-5 mEq/L of potassium is in plasma. Nonetheless, the relatively small amount of K^+ that resides in ECF compartment has major clinical implications; therefore, serum K^+ concentration is tightly regulated by body. Most of the potassium is obtained from diet and is excreted from the distal tubule in exchange of sodium under the influence of aldosterone. Potassium is important for maintenance of intracellular osmotic pressure and plays important role in functions of neuromuscular junctions and contractility of muscles. Extracellular potassium is a major determinant of the cell resting membrane potential.

Potassium deficit/Hypokalaemia

Hypokalaemia is a condition when serum K^+ concentration is less than normal (i.e. <3.5 mEq/L). Hypokalaemia occurs relatively frequently in domestic animals and may result from decreased K^+ intake, dilution of blood potassium with inappropriate fluid therapy, redistribution of potassium from ECF into ICF space, and/or excessive loss from body. Dietary deficiency alone is not a common cause of potassium deficit because potassium is present in relatively high concentrations in most animal feeds. Fluid therapy with inadequate K^+ supplementation (e.g. lactated Ringer's solution) may dilute blood concentration, but again is not an important cause of hypokalaemia. Hypokalaemia most often is associated with excessive potassium loss either from GI tract as a result of vomiting or diarrhoea, or from kidney as a result of certain diuretics, excess mineralocorticoid or altered renal tubular functions. Intracellular movement of potassium from the ECF space may also develop hypokalaemia, particularly during an acute alkalosis and in patients treated with insulin, and glucose and sodium bicarbonate infusions.

Potassium depletion may result in decreased ICF volume, altered intracellular pH and modified potassium-dependent enzymatically mediated reactions. Hypokalaemia increases resting membrane potential, resulting in a larger difference between the resting potential and the threshold potential necessary for an action potential and muscular contraction. The clinical features of hypokalaemia depend on the state of total body potassium content and also on the rate with which hypokalaemia develops. Acute hypokalaemia can produce a much greater alteration in membrane potential and thus more marked clinical signs than a gradually developing hypokalaemia of the same magnitude. Important clinical features include muscle weakness, ileus, cardiac arrhythmias, rhabdomyolysis and renal dysfunction.

Treatment: Specific treatment of hypokalaemia in animals includes oral or parenteral replacement therapy. Although oral route is the preferred way to administer potassium (e.g. potassium chloride or potassium gluconate), some patients require parenteral therapy (usually administered in IV maintenance fluids) because of vomiting and anorexia. Potassium chloride solution is preferred

to other salts of potassium because many patients have concurrent alkalosis and chloride deficiency. Potassium phosphate can also be used, but care should be taken not to produce hyperphosphataemia and subsequent hypocalcaemia. Potassium should never be administered at a rate greater than 0.5 mEq/kg/hour by IV route because too rapid administration of potassium may produce fatal cardiotoxic effect.

Potassium excess/Hyperkalaemia

Hyperkalaemia is a condition when serum potassium concentration is more than normal (i.e. > 6 mEq/L). Potassium excess is seen relatively rarely in animals. Hyperkalaemia in animals may occur due to increased intake, decreased excretion and/or compartmental shifts of potassium from cells to ECF. Increased intake of potassium does not occur naturally in animals, but is mainly iatrogenic (administration of potassium containing drugs). Potassium excess in animals occurs mainly as a consequence of some defect in renal excretion of potassium usually seen in conditions such as Addison's disease, acute renal failure and renal shutdown secondary to inadequate effective circulatory fluid volume. Shifts of potassium from ICF to ECF compartments are not important cause of hyperkalaemia but occasionally acidosis and some drugs (e.g. beta-adrenoceptor blockers and digitalis) may produce hyperkalaemia by this mechanism. Sometimes excessive tissue destruction (e.g. tumour lysis syndrome) may release considerable amount of potassium into the blood; spontaneous lysis of emboli or haemolysis may produce the same result.

Hyperkalaemia often increases the resting membrane potential to near threshold, which produces weaker action potential resulting in muscle weakness. Patients with potassium concentration greater than 7.5 mEq/L usually exhibit clinical signs of hyperkalaemia. Important symptoms due to muscle weakness include reluctance or inability to stand and walk, tremors, bradycardia and cardiac arrhythmia. Hyperkalaemia often produces abnormal ECG with diminution of P waves, prolongation of P-R interval, widening of QRS complex and diminished R wave amplitude. Hyponatraemia, hypocalcaemia and acidaemia potentiate effects of hyperkalaemia on the heart.

Treatment: Treatment of hyperkalaemia includes management of underlying cause, withholding of potassium rich foods and administration of potassium free fluids. Fluid replacement is usually indicated in hyperkalaemia because most patients are dehydrated. Administration of potassium free fluids causes dilution of K^+ and increases renal blood flow, which in turn increases K^+ excretion. Sodium bicarbonate administration may also be done in severe cases to increase blood pH and to cause a shift of K^+ into cells. Calcium (e.g. calcium gluconate or calcium chloride) may be administered by slow IV route to counter the cardiotoxic effect of K^+ until other therapy can decrease blood potassium values. Insulin plus glucose may also be employed to deposit K^+ with glycogen intracellularly in the liver and muscle. Administration of glucose alone may also resolve hyperkalaemia but it usually requires more time (>1 hour) before endogenous insulin release produces effect on K^+.

Increased loss of K^+ from the body may be promoted by careful use of diuretics (e.g. thiazides and loop diuretics). However in patients with hyperkalaemia due to primary renal disease, such medications are usually ineffective. In these patients, K^+ must be removed via the exchange resins (sodium polystyrene sulphonate mixed with water, orally in divided doses), peritoneal dialysis and/or haemodialysis.

Chloride

Chloride is an anion that combines with sodium to form sodium chloride (salt). It generally follows sodium in the physiological processes which regulate body fluid and electrolyte balance. Changes in the chloride concentration which are not associated with a similar change in sodium concentration are usually associated with acid-base balance. Chloride concentration tends to vary inversely with bicarbonate concentration.

Chloride deficit/Hypochloraemia

Hypochloraemia occasionally develops in animals along with hyponatraemia due to either excessive loss or sequestration of fluids. The major cause of hypochloraemia is increased chloride loss due to vomiting (chloride losses due to vomiting are usually associated with hypokalaemia and metabolic alkalosis) or excessive administration of loop diuretics. Rarely, administration of large doses of sodium without administration of chloride (e.g. sodium salts of drugs) may cause hypochloraemia.

Treatment: Administration of isotonic saline solution (0.9% NaCl) with or without potassium chloride is generally considered adequate to replace chloride in hypochloraemia and resolve the problem.

Chloride excess/Hyperchloraemia

Hyperchloraemia is mainly found in animals those are hypernatraemic due to loss of free water and in animals which have received fluids containing proportionally more chloride than sodium relative to plasma values (e.g. Ringer's solution, physiological saline solution supplemented with potassium chloride). Hyperchloraemia may also be present in animals which have a metabolic acidosis associated with a normal anion gap (i.e. hyperchloraemic metabolic acidosis). Chloride concentration increases in this type of acidosis as a result of proportionately smaller losses of chloride than bicarbonate and enhanced renal chloride resorption in response to decreased bicarbonate.

Treatment: Treatment of hyperchloraemia due to free water loss is usually done by administration of dextrose 5% in water (D5W). If the animal has a corrected hyperchloraemia due to hypoadrenocortism, the patient is treated with isotonic normal saline (0.9% NaCl) and steroids, and not with D5W.

Calcium

Calcium is an important electrolyte that is a major component of bones and teeth, and is required for maintenance of a normal cardiac rhythm, blood clotting and initiation of neuromuscular and metabolic activities. Movement of the calcium ion into and out of the cytoplasm also functions as a signal for many cellular processes. Total serum calcium consists of three fractions: biologically active ionised calcium (~50%), protein bound calcium (~40) and complexed calcium (~10%). Total serum calcium are variable but may range between 10-11 mg/dL in dogs and about 9 mg/dL in cats. Ionised calcium is important for normal physiological and pathological activity. Homeostasis of calcium is mainly regulated by parathyroid hormone, calcitonin and vitamin D.

Calcium deficit/Hypocalcaemia

Symptomatic hypocalcaemia is mainly caused by puerperal tetany, hypoparathyroidism, ethylene glycol intoxication or inappropriate administration of a hypertonic phosphate enema. In dairy cows, short-term hypocalcaemia almost always occurs at or soon after parturition when mammary gland secretions exceed the animal's requirement for calcium. In beef cattle and sheep,

hypocalcaemia may occur when animals are fed a diet high in oxalate containing plants such as *Oxalis* species.

Clinical signs of hypocalcaemia in cattle are characterised by an initial short period of excitement, muscle tremors and a stiff gait followed by muscular weakness leading to sternal recumbency.

Treatment: Hypocalcaemia causing clinical signs should be treated with calcium chloride or calcium gluconate. Calcium chloride produces faster action than calcium gluconate but it causes more adverse effects like severe sloughing if injected perivascularly and cardiotoxicity if injected rapidly. Once the severe clinical signs are over, calcium may be added to the IV fluid to provide constant infusion. In general, 10 ml of 10% calcium gluconate is added to 500 ml of isotonic normal saline (0.9% NaCl) and given at maintenance rates. In ruminants either borogluconate 20% or calcium borogluconate 40% is administered by slow IV infusion while monitoring the heart rate and rhythm.

Calcium excess/Hypercalcaemia

Elevated serum calcium occurs relatively uncommonly in dogs. Symptomatic hypercalcaemia in dogs may occur due to hyperparathyroidism, hypoadrenocortism, vitamin D intoxication, chronic renal disease or fluid infusion containing calcium supplementation. Increased concentration of ionised calcium is deleterious to the integrity of cellular function as it causes alterations in cell membrane permeability and cell membrane pump activity. Increased cellular content ultimately can result in cell death due to deranged cell function and reduced energy production. Although all tissues may be affected by hypercalcaemia, effects on the central nervous system, heart and kidneys are of most clinical importance. Mineralisation of soft tissues is an important sequel to hypercalcaemia, particularly in the heart and kidneys.

Important clinical signs of hypercalcaemia include anorexia, vomiting and constipation. Generalised weakness due to decreased excitability of skeletal muscle and CNS depression are common in severe hypercalcaemia. Rarely stupor, coma and seizures can be observed.

Treatment: Treatment of hypercalcaemia includes management of primary cause and normalisation of plasma concentration of calcium to prevent nephrotoxicity. Administration of isotonic saline (0.9% NaCl) or loop diuretics initiates saline diuresis and enhance urinary calcium excretion. However, potassium concentration should be monitored carefully to avoid hypokalaemia. Administration of glucocorticoids (e.g. prednisolone) may also enhance calcium excretion and reduce bone resorption as well as intestinal calcium absorption. In severe cases, one may also alkalinise the patient with sodium bicarbonate or administer prednisolone, mithramycin, calcitonin or combination therapy.

Magnesium

Magnesium is an essential electrolyte that acts as a cofactor in numerous enzyme systems and is involved in muscle contractility and neuronal transmission. Magnesium ions also play major roles in manipulating important biological polyphosphate compounds like ATP, DNA and RNA. About 50 percent of total body magnesium exists in insoluble state in bones, about 5 percent in present as extracellular cation and remaining 45 percent is contained in cells as an intracellular cation. The normal plasma level of Mg^{++} is 1.5-2.5 mEq/L, out of which one-third is bound to protein and two-thirds is in a free state. Excretion of Mg^{++} is mainly via the kidney.

Magnesium deficit/ Hypomagnesaemia

Hypomagnesaemia is occasionally reported in ruminants, which feed on lush pasture associated with heavy fertilizer application containing nitrogen and potash. It is also reported in cows exposed to adverse winter conditions. Renal and gastrointestinal losses, starvation, malabsorption, hypoparathyroidism, etc. are other probable causes of hypomagnesaemia. Acute hypomagnesaemia is associated with CNS hyperirritability. It causes ataxia, excitability and collapse with titanic convulsions of limbs and neck. Chronic magnesium deficiency may be associated with decreased appetite and reduction in milk yield.

Treatment: Treatment of hypomagnesaemia includes administration of parenteral fluids containing magnesium chloride or sulphate. Magnesium salts may be administered as a 20% (or lower) solution in D5W. As hypomagnesaemic tetany is mainly due to long-term dietary magnesium deficiency, it is essential to ensure adequate daily magnesium supplementation, particularly in lactating cows.

Magnesium excess/Hypermagnesaemia

Magnesium excess is reported rarely in animals. It may result from renal insufficiency and inability of kidneys to excrete magnesium. Occasionally excessive intake of magnesium (e.g. magnesium sulphate as cathartic), especially by patients with renal dysfunction may produce hypermagnesaemia.

Treatment: Calcium salts are used in hypermagnesaemia to counteract the respiratory effects. Haemodialysis or peritoneal dialysis is indicated in severe hypermagnesaemia with co-existing renal insufficiency.

Phosphorus

Phosphorus is an important component in energy and protein metabolism and also as a structural part of bone. Phosphorus homeostasis is controlled by vitamin D and calcium. Acute phosphorus deficiency is rarely seen in animals. Phosphorus deficiency in lactating cows has been associated with development of post-parturient haemoglobinuria after the onset of acute haemolysis. More commonly phosphorus deficiency causes chronic hypophosphataemia, which may result in skeletal defects, lameness and low milk production. Anorexia, pica, impaired weight gain and infertility may also occur with chronic phosphorus deficiency.

Treatment: Treatment of acute hypophosphataemia with or without hypocalcaemia should be treated by IV injection of phosphorus containing solutions. For dietary supplementation, it is usual to supply phosphate along with calcium in the ratio of 1.5 or 2 parts calcium to 1 part phosphorus.

ACID-BASE IMBALANCE

Acid-base imbalance is an abnormality in which the normal equilibrium between the acids and bases in body is disturbed and the plasma pH is deviated out of the normal range (7.35 to 7.45). Disturbance in acid-base balance is clinically very important because it adversely affects functioning of various body systems. In general, pH values of arterial blood below 7.4 are classified as acidosis and pH values above 7.4 are classified as alkalosis. These changes are mainly the result of either respiratory or metabolic abnormalities, which disturb the normal 20:1 HCO_3^- :

H_2CO_3 ratio. A pH imbalance caused by a change in CO_2 levels is called respiratory acidosis or respiratory alkalosis depending on whether the CO_2 level is increased or decreased, respectively. Similarly, a pH imbalance caused by a change in HCO_3^- level is called metabolic acidosis or metabolic alkalosis depending on whether the HCO_3^- level is decreased or increased, respectively. In such acid-base imbalance conditions, whenever possible, the body attempts to compensate for a pH imbalance by adjusting the activities of lungs and/or kidneys. The four primary acid-base imbalances and their compensating responses are presented in Table 40.2.

Acidosis

Acidosis is a process that causes acid (H^+) to accumulate in body that tends to decrease the pH of extracellular fluids. If not further described, it usually refers to acidity of the blood plasma, especially when arterial blood pH falls below 7.35 (except in the foetus). Acidosis may occur as a result of respiratory or metabolic abnormalities and is produced by either an excess of hydrogen ion or a deficit of bicarbonate ion.

Respiratory acidosis

Respiratory acidosis occurs due to a depressed respiration (hypoventilation), which causes accumulation of carbon dioxide in body. It is characterised by an elevation of pCO_2 and a decrease in blood pH. The increase in blood CO_2 results in more synthesis of H_2CO_3 and subsequently more of hydrogen ion in blood. The increase in blood CO_2 causes a fall in the normal 20: 1 HCO_3^- : H_2CO_3 ratio and thus a depression of the blood pH. Blood gas analysis under these conditions, may reveal an elevated arterial pCO_2, a decrease in pH and a little increase in blood HCO_3^- (due to more production from H_2CO_3 i.e. $H_2CO_3 \rightarrow H^+ + HCO_3^-$). Respiratory acidosis is not commonly seen in animals except for animals which are not being properly ventilated while under anaesthesia. Respiratory acidosis may be caused by any disease or drug, which affects respiration. These include acute respiratory obstruction, pneumonia, pneumothorax, asthma, chronic obstructive lung disease, respiratory centre paralysis, tetanus, seizures, general anaesthetics and opioids. Head injuries and brain tumours which affect respiration may also cause respiratory acidosis. Respiratory acidosis can also occur as a compensatory response to chronic metabolic alkalosis.

Clinical signs of acidosis are related to elevation of pCO_2 that causes vasodilatation and an increase in cerebral blood flow resulting in CNS signs including anxiety, depression and altered CNS functions. Hypercapnia also stimulates the sympathetic nervous system, causing tachycardia, sweating and elevated body temperature. Elevated pCO_2 also causes dilation of peripheral blood vessels, imparting a 'bright red' colour to mucous membrane, and sensitises heart to produce arrhythmias. The compensating response for a respiratory acidosis includes renal secretion of hydrogen ions and regeneration and resorption of bicarbonate ions. This response requires several days and hence the compensating response is only seen in a chronic respiratory acidosis. When respiratory acidosis is chronic, blood gas analysis may reveal high HCO_3^-, elevated pCO_2 and a small decrease in pH.

Treatment: Treatment of respiratory acidosis depends on the underlying cause. In acute conditions, ventilation must be supported by mechanical means, bronchodilators, endotracheal intubation, etc. The pCO_2, pO_2 and pH of arterial blood should be monitored carefully and regularly. Treatment of the primary cause is utmost essential for the complete recovery. Any change in pCO_2 should be made gradually to avoid seizures and cardiac arrhythmias. The patient should not be treated with high-inspired O_2 concentration unless hypoxia is life threatening because this may abolish the ventilatory drive (hypoxemia is main stimulus to ventilation) and result in an acute rise in pCO_2. Use of sodium bicarbonate is contraindicated in respiratory acidosis because this generally enhances production of CO_2, which may not be eliminated efficiently by the animal. The retained CO_2 can also enter cells and readily produce a more severe intracellular acidosis. Treatment of chronic respiratory acidosis is more difficult and more challenging than the acute respiratory acidosis.

Metabolic acidosis

Metabolic acidosis occurs mainly due to either an addition of more hydrogen ions or a loss of bicarbonate ions. Metabolic acidosis, irrespective of the cause (high H^+ or low HCO_3^-), is characterised by a decrease in blood bicarbonate concentration, in contravention to respiratory acidosis where the bicarbonate is either normal (uncompensated) or increased (compensated). A fall in HCO_3^- concentration, either by excess HCO_3^- loss or H^+ buffering action, (excess hydrogen ions donated by an acid are buffered by bicarbonate), produces a fall in the normal 20: 1 HCO_3^-/H_2CO_3 ratio, thus depressing the pH. Metabolic acidosis is the most common acid-base balance disturbance encountered in small animal practice. The common causes include lactic acidosis, keto-acidosis, diabetes mellitus, gastrointestinal loss of bicarbonate due to diarrhoea, and renal failure that results in a decrease ability to excrete hydrogen ion. Additional causes of metabolic acidosis include ingestion of certain drugs or toxicants such as salicylates, methanol, ethylene glycol or paraldehyde that results in accumulation of exogenous anions. Acid consumption from poisoning, increase levels of iron in the blood and chronically less production of bicarbonate may also produce metabolic acidosis.

Clinical signs of metabolic acidosis depend on the primary causative disease. Central nervous system signs are mostly absent even in severe metabolic acidosis because hydrogen ions cross the blood brain barrier slowly and the CSF has considerable buffering action. This is in contrast to respiratory acidosis where CO_2 easily enters brain and produces acute CSF acidosis and altered CNS functions. Metabolic acidosis may produce a direct depressant action on cardiac contractility and output. A profound metabolic acidosis without dehydration leading to depression, recumbency and death has been seen in goat kids and calves.

The compensatory response of a metabolic acidosis occurs by hyperventilation and reduction of pCO_2, which in turn causes reduction of H_2CO_3 in extracellular fluid. Unlike respiratory acidosis, metabolic acidosis is recognised very quickly by body and compensatory response of increased ventilation starts within minutes. However, the compensatory responses are short-lived, lasting only a few days. Long-term correction of a metabolic acidosis requires renal bicarbonate retention and enhanced renal H^+ excretion. Laboratory findings would normally reveal a low pH, a low arterial pCO_2 owing to respiratory compensation and increase bicarbonate due to renal retention.

Treatment: Treatment of metabolic acidosis essentially requires correction of the primary metabolic defect (e.g. control of diabetes mellitus and treatment of diarrhoea). Specific treatment of hydrogen ion imbalance should only be instituted when metabolic acidosis is severe (i.e. arterial blood pH <7.2) and can

no longer be compensated for adequately by the lungs. Water is replenished and deficit of Na^+, K^+, HCO_3^- and other electrolytes should be made. Treatment of severe metabolic acidosis requires use of alkalinising agent, preferably sodium bicarbonate solution. Synthetic buffers such as tromethamine (THAM; tris buffer) are not widely used. The goal of sodium bicarbonate therapy should be to raise the pH to approximately 7.2 or slightly more, but not to correct the pH back into the normal range. Administration of too much alkali initially may enhance the pH above normal.

Alkalosis

Alkalosis is a process that causes acid (H^+) to decrease in body. If not further described, it usually refers to alkalinity of the blood plasma, especially when arterial blood pH exceeds 7.45. Alkalosis may occur as result of respiratory or metabolic abnormalities and is produced either by a deficit of hydrogen ion or an excess of bicarbonate ion.

Respiratory alkalosis

Respiratory alkalosis occurs as a result of hyperventilation, which causes increased elimination of carbon dioxide from the body. The decrease in pCO_2 causes a rise in the normal 20: 1 HCO_3^- : H_2CO_3 ratio and thus an elevation of the pH. Respiratory alkalosis is relatively common in animals and may occur due to any cause that stimulates respiration. These include excitement, fear, pain, psychogenic hyperventilation, mechanical respirators, stimulation of respiratory centre by encephalitis, hypoxia and salicylate poisoning. Ventilation may also be stimulated by hypoxemia associated with pulmonary disease, congestive heart failure or severe anaemia.

Clinical signs of respiratory alkalosis are related to the decreased blood carbon dioxide levels and include peripheral paraesthesia, hyperpnoea, hyperactive tendon reflexes and CNS stimulation. In addition, the alkalosis may disrupt calcium ion balance and cause signs of hypocalcaemia (such as tetany and fainting) with no fall in total serum calcium levels.

The initial compensating response to an acute respiratory alkalosis is modest decline in ECF bicarbonate concentration as a result of an efflux of H^+ ions from cells into ECF where they react with HCO_3^-, producing a buffering action. Subsequent renal responses result in decreased ECF bicarbonate concentration through reduced renal bicarbonate reabsorption. The decline in bicarbonate is partially offset by chloride retention in order to retain electro-neutrality. In the acute stages, blood gas analysis may reveal a low pCO_2 and a high pH, but bicarbonate levels are normal. When renal compensatory mechanisms come into play (2-3 days), bicarbonate levels are low and there is negative base excess (compensatory metabolic acidosis). Other laboratory findings include an increase in plasma chloride and a slight fall in plasma potassium.

Treatment: Treatment of respiratory alkalosis involves normalisation of ventilation and removal of primary cause, if any. If fever or heat stress is the primary cause of hyperventilation, reduction of body temperature to normal may correct the hyperventilation. Oxygen therapy may be used if hyperventilation is due to hypoxic drive. In case of pain, fear, anxiety and psychogenic hyperventilation, judicious use of analgesics and/or tranquillizers are effective. The use of mechanical respirators should be based on the pCO_2 and pH values of arterial blood.

Metabolic alkalosis

Metabolic alkalosis occurs when blood pH is elevated either by excessive loss of H^+ ions or from excessive administration of bicarbonate. The increase in bicarbonate or loss of H^+ causes a rise in normal 20: 1 HCO_3^- : H_2CO_3 ratio, and thus an elevation of blood pH. Metabolic alkalosis with dehydration is often caused in small animals by excessive loss of hydrochloric acid due to vomiting. In ruminants, it may be caused by sequestration of chloride rich fluid in the abomasum and fore stomach. Excessive renal hydrogen loss associated with mineralocorticoid excess, diuretic therapy (e.g. loop diuretics) and low chloride intake may cause or contribute to the generation of a metabolic alkalosis. Hydrogen movement into cell in response to potassium depletion and excessive bicarbonate or other alkalinising agents (e.g. lactate) may also produce metabolic alkalosis. Blood gas analysis generally reveals an elevated pH and plasma HCO_3^- values. The pCO_2 is usually normal or slightly elevated.

Clinical signs of metabolic alkalosis include depressed breathing (slow and shallow), nervous excitement including tetany and convulsions, and muscular hyperactivity. Signs of dehydration may be present in cases of acute gastric vomiting.

The compensatory response to metabolic alkalosis includes the initial buffering action by H^+ ions derived from intracellular proteins and phosphates and reduced alveolar ventilation which raises pCO_2. However, these compensatory responses are small and inadequate. Further, renal compensation of metabolic alkalosis is also inadequate because in conditions like vomiting (loss of H^+, Cl^- and water), body tries to retain HCO_3^- and cannot adequately excrete H^+. In such conditions, hydrogen and potassium ions are excreted in exchange for the sodium ions (Na^+ is reabsorbed to restore ECF) and HCO_3^- accompany the sodium ions (HCO_3^- is retained along with Na^+ because there is a paucity of Cl^- ions). A similar pathophysiology occurs when excessive loop diuretics therapy results in vigorous diuresis with chloride rich urine. The role of potassium in metabolic alkalosis is complex, but hypokalaemia is more often a result of metabolic alkalosis than a cause.

Treatment: Metabolic alkalosis usually remains uncompensated until the primary cause is treated and the mechanisms causing excessive renal HCO_3^- reabsorption are reversed. Metabolic alkalosis induced by conditions like vomiting and diuretics are considered as chloride responsive alkalosis because administration of chloride resolves the problem. Correction of volume depletion and supplementation with chloride are most often done with 0.9% sodium chloride solution. Severe hypokalaemia should be corrected by the addition of potassium chloride to intravenous fluids. Acidifying agents like ammonium chloride may be administered to correct specifically hypochloraemic alkalosis caused by mercurial diuretics.

Mixed acid-base imbalance

A mixed acid-base balance is the co-existence of two or more types of acid-base disorders in the same patient. Metabolic acidosis and alkalosis can co-exist and either one or sometimes both of these metabolic abnormalities may occur with respiratory acidosis or alkalosis. Mixed disorders featuring an acidosis and alkalosis at the same time may partially counteract each other, or there can be two different conditions affecting the pH in the same direction. Evaluation of mixed acid-base abnormalities requires careful observation of clinical situations, repeated laboratory

determinations, an understanding the anion gap and limits of compensation for acid-base imbalances. Recognition of mixed acid-base disturbances is often of therapeutic value because treatment of one type of acid-base disorder without considering other type will convert one type of disturbance into another type.

Table 40.2: Acid-base imbalance and compensatory responses.

Disorder	Blood pH	Primary Imbalance		Compensatory Response	
		pCO_2	$[HCO_3^-]$	pCO_2	$[HCO_3^-]$
Respiratory acidosis	↓	↑	–	–	↑
Metabolic acidosis	↓	–	↓	↓	–
Respiratory alkalosis	↑	↓	–	–	↓
Metabolic alkalosis	↑	–	↑	↑	–

↑ = Increase; ↓ = decrease; – = no change.

SPECIAL CONSIDERATIONS

Hypovolaemic shock

Shock may be defined as a state of underperfusion of tissues, especially vital tissues. Hypovolaemic shock is a type of shock occurring due to decreased blood volume, more specifically, decrease in volume of blood plasma. In clinical conditions, hypovolaemic shock occurs as a result of either severe dehydration (loss of water) or haemorrhage (loss of whole blood). Reduction in blood volume may lead to acute circulatory failure in which cardiac output is not adequate to maintain peripheral perfusion and oxygenation. Important clinical manifestations of hypovolaemic shock are elevated pulse, diminished blood pressure, tachycardia, cold and mottled skin, dry mouth, increased thirst, hypothermia and absence of perfusion as assessed by skin signs (skin turning pale) and/or capillary refill. This type of shock can cause many organs to stop working.

In haemorrhagic shock, the optimal therapy is administration of fresh blood. Alternatively, plasma or balanced crystalloids (e.g. lactated Ringer solution) may be used. Other options are colloids (e.g. dextran 70 or hetastarch) or hypertonic crystalloids (hypertonic 7% saline solution). Hypertonic saline solution should be followed up with a reduced rate of isotonic crystalloids. Hypertonic saline solution should be used cautiously or even avoided in patients those are already hypernatraemic or have cardiogenic shock.

Heat stroke

Heat stroke is a form of hyperthermia, an abnormally elevated body temperature with accompanying physical and neurological symptoms. The heat stroke is often associated with lack of thermoregulation and thus is different from fever where there is a physiological increase in the temperature set point of the body. Heat stroke occurs when a patient is exposed to excessive heat and has free water loss resulting in severe hypernatraemic dehydration. This dehydration can affect the vital organs and contribute to renal failure, GI hypoxia and disseminated intravascular coagulation. Heat stroke is a medical emergency that can be fatal if not properly and promptly treated.

The patient suffering from heat stroke should be cooled off quickly via cold-water sprays, enemas, IV fluids or a combination therapy. The depleted circulatory volume should be restored with fluid therapy initially with almost shock doses. It is usually started with isotonic half-strength physiological saline solution (0.45% NaCl) plus 2.5% dextrose and then followed by isotonic saline solution (0.9% NaCl) or lactated Ringer solution.

Vomiting

Vomiting is the forceful expulsion of the gastric contents through the mouth and sometimes the nose. Prolonged and excessive vomiting often depletes the body water and may alter the electrolyte status. The severity of water and electrolyte loss depends on the severity, duration and cause of vomiting and on whether the animal takes food or water. Gastric vomiting leads to the loss of acid (protons) and chlorine directly. Combined with the resulting alkaline tide, this leads to hypochloraemic metabolic alkalosis and often hypokalaemia. A less frequent occurrence results from a vomiting of intestinal contents, including bile acids and HCO_3^- which can lead to metabolic acidosis.

Control of vomiting involves treatment of primary cause associated with use of appropriate anti-emetic drug and compensation of water and electrolyte loss. If mild dehydration is present or anticipated, SC fluids are often considered adequate. If the dehydration is severe or if the animal appears to be going into shock, the IV fluids are mandatory until the electrolyte and acid-base status are known. In such cases, isotonic saline solution or lactated Ringer solution is usually administered. Hypokalaemia, if present, should be treated carefully.

Diarrhoea

Diarrhoea is the frequent passage of loose or liquid faeces. Acute diarrhoea often causes dehydration and loss of electrolytes. In secretory diarrhoea, there is secretion of anions, especially chloride ions; to maintain a charge balance in the lumen, sodium is carried with chloride along with water. In osmotic diarrhoea, too much water is drawn into the bowels. In many cases of diarrhoea, replacing the lost fluid and salts is the only treatment needed. In less severe cases, fluid and electrolyte loss can be easily compensated by instituting oral rehydration therapy. Severe diarrhoea, if associated with vomiting and anorexia, essentially requires IV fluid therapy. Treatment of diarrhoea in such cases should be started with lactated Ringer solution. It is also useful to administer isotonic normal saline supplemented with 20 mEq KCl/L as maintenance fluid.

Ketosis

Ketosis is a state characterised by elevated levels of ketone bodies in the blood. Ketone bodies are formed from breakdown of fatty acids by the process of ketogenesis, especially when the liver glycogen stores are depleted. In dairy cattle, primary ketosis results from high glucose demand of the mammary gland for lactose synthesis. This increased requirement for glucose causes a decreased amount of available oxaloacetate for the oxidative metabolism of acetate via the Krebs cycle. This results in accumulation of ketone bodies like acetoacetate, betahydroxybutyrate and acetone in blood, milk and urine. In the early post-partum period, a reduced appetite secondary to mastitis, metritis or left abomasal displacement may cause secondary ketosis resulting from poor appetite.

Treatment of dairy cattle with ketosis should be started by providing glucose, generally by IV injection of dextrose 50% solution. In addition, glucose precursors such as propionate, lactate, glycol or propylene glycol should be given by mouth. Feeding an appropriate diet to supply sufficient dietary energy

during the late gestation can control ovine pregnancy toxaemia and starvation ketosis in cattle.

FLUID AND ELECTROLYTE THERAPY

Commercially, two major categories of fluids are available for clinical use – crystalloids and colloids. A crystalloid fluid is a water-based solution with small particles which are normally osmotically active in body fluids and can pass through the capillary membranes. In contrast, colloid fluids contain large particles which cannot pass through the capillary membranes and thus do not readily leave the intravascular space, but attract and hold water in the vascular space thereby expanding vascular volume and exerting osmotic pressure in a manner similar to that of plasma proteins. Crystalloids are more commonly utilised for fluid therapy than colloids and will be discussed in the present chapter (see for colloids Chapter 37).

Crystalloid Solutions

Crystalloid solutions are mostly sodium based electrolyte solutions with or without glucose in water. These solutions provide water, electrolyte and, in some cases, alkalinising agents and/or calorie. The crystalloid solutions are widely used in therapeutics because they are effective both as replacement and maintenance fluids, easy to store with long shelf-life, readily available, inexpensive and produce very low incidence of adverse effects. Further, there are a variety of crystalloid preparations on the market, varying in composition and osmolality. These crystalloid solutions are used clinically as replacement and/or maintenance fluids. Some crystalloid solutions are used also for specific purposes.

Replacement fluids: Replacement fluids are mainly isotonic sodium based fluids which have composition similar to that of plasma, and which when given rapidly in large volumes do not drastically change constituents of intravascular fluid.

Maintenance fluids: Maintenance fluids are used as substitutes for normal intake of water and dietary electrolytes and are provided after initial fluid balance is restored. These may be used either by parenteral route or by oral route.

I. Parenteral Fluid Preparations

A large number of parenteral fluid preparations are used in both human and veterinary medicines. Some of these parenteral fluid preparations are discussed below and some others are summarised in Table 40.3.

1. Saline Solutions/Sodium Chloride Solutions

Isotonic saline solution/Normal saline solution

Isotonic saline solution (Normal saline solution, physiological saline solution, isotonic sodium chloride solution) contains 0.9 percent w/v sodium chloride in water. It is one of the most widely used replacement fluids, which is occasionally also used as a maintenance fluid.

Isotonic saline solution is mildly acidic in nature. It is often called normal or physiological saline despite the fact that its chloride content is much higher than that of blood. However, as it contains approximately 300 mOsm per kg of fluid, it is isosmotic with plasma. When isotonic saline solution is given IV, almost all of it stays in the ECF. In the ECF, about two third distributes to the interstitial space and one-third remains in the intravascular space. As its sodium concentration is higher than that of plasma, it replaces sodium loss efficiently.

Although isotonic saline solution is acceptable as an isotonic replacement fluid, it is not ideal in most situations because it is not a balanced fluid (balanced fluid should contain electrolyte composition approximately similar to that of extracellular fluid). As it contains higher amount of chloride than plasma, it can cause a hyperchloraemic metabolic alkalosis, especially if a large amount is given to a patient with diarrhoea that has lost more sodium than chloride or if the kidney is unable to excrete the excess chloride. Isotonic saline solution should not be used in animals with borderline congestive heart failure or in other sodium-restricted conditions unless there is marked hypovolaemia or a condition of severe total body sodium depletion. Isotonic saline solution is also used as maintenance fluid, but its long-term use is not advised unless there is heavy and continued loss of electrolytes. It is frequently used for irrigation of body cavities and in cell biology, molecular biology and biochemistry experiments. Despite several limitations, isotonic saline solution remains a widely used electrolyte solution in veterinary medicine because it is cheap and readily available.

Dose

Dogs & cats : 40 – 50 ml/kg/day, IV infusion, SC, or IP.

Hypertonic saline solution/Hypertonic sodium chloride solution

Hypertonic saline solutions are available commercially as 3%, 5% and 7.5% w/v sodium chloride in water. As hypertonic saline solutions have significantly more osmotically active particles per unit of volume or weight compared with the intracellular fluid, they cause water movement from the interstitial space.

Hypertonic saline solutions are considered beneficial in the early treatment of hypovolaemia and haemorrhagic shock in man and animals as they rapidly expand the blood volume and help in maintaining perfusion. In the management of severe shock, in addition to hypovolaemic resuscitation, hypertonic saline may provide a beneficial mild positive inotropic effect and produce systemic and pulmonary vasodilatation. They are also indicated in conditions where there is severe sodium ion loss in association with relative water excess (hypervolaemic-hyponatraemia) or when there is primary water excess (water intoxication or syndrome of inappropriate antidiuretic hormone secretion).

Hypertonic saline solution alone is contraindicated in patients those are dehydrated, hypernatraemic or hyperchloraemic. Its use should also be avoided in critically ill patients with little tolerance for interstitial volume increases (e.g. cardiac dysfunction and neurological derangements). Hypertonic saline solutions should be given only IV and should be given slowly because rapid infusion can result in vagally mediated hypotension and bradycardia, bronchoconstriction, and rapid and shallow breathing. Hypertonic saline increases preload for only about 30 minutes; therefore it is important to administer appropriate volumes of fluid or colloid following hypertonic saline so as to maintain the improvement.

Dose

5.0% Saline solution (5% sodium chloride solution)

Dogs : 6 – 10 ml/kg, IV infusion at a maximum rate of 1 ml/kg/min.

7.5% Saline solution (7.5% sodium chloride solution)

Dogs : 4 – 8 ml/kg, slow IV infusion at a maximum rate of 1 ml/kg/min. This is to be followed by balanced crystalloid.

Cats : 2 – 6 ml/kg, IV infusion; to be followed by balanced crystalloids.

Hypotonic saline solution/Hypotonic sodium chloride solution

Hypotonic saline solution contains 0.45 percent sodium chloride in water. As the salt concentration of hypotonic saline is half of the isotonic saline solution, it is also called as half-normal saline solution. Hypotonic saline solution has an osmolality less than that of extracellular fluid.

After hypotonic saline solution is administration by IV route, one-third of the volume goes into the intracellular fluid and about two third remains in the extracellular fluid. Hypotonic saline solution is mainly used as a maintenance fluid, often in combination with 2.5% dextrose. Half-normal saline with 2.5 percent dextrose is considered as the fluid of choice for patients those have sodium restrictions and those tend to develop hypernatraemia.

Other sodium chloride solutions

Some other less frequently used parenteral solutions of sodium chloride are also available commercially and are employed for specific purposes. These include quarter-normal saline (0.22% NaCl) having 39 mEq/L of Na and Cl; it always contains 5% dextrose for osmolality reasons. A 0.18% sodium chloride solution is used sometimes for maintenance replacement. A highly hypertonic saline solution containing 23.4% sodium chloride (approx 4000 mEq/L) is used via central line, often in conjunction with supplementary diuretics, in the treatment of traumatic brain injury.

2. Ringer Solutions

Ringer solution

Ringer solution is the prototype replacement fluid that contains sodium, potassium, calcium and chloride in appropriate concentrations to make it an isotonic electrolyte solution. It was invented by Sydney Ringer, a British clinician and pharmacologist. Although it contains balanced electrolytes and has an osmolality of about 310 mOsm/L, it has high chloride content and has no precursor of bicarbonate. Occasionally, it is also used as maintenance fluid.

Dose

Dogs & cats : 40 – 50 ml/kg/day, IV, SC, or IP for maintenance.

Lactated Ringer's solution

Lactated Ringer's solution (Compound sodium lactate solution, Hartmann's solution) is a Ringer's solution that contains additional lactate, a precursor of bicarbonate. Bicarbonate as such is not used in Ringer's solution because it decomposes on autoclaving to give carbonate that may react with calcium to form precipitates of calcium carbonate. Low chloride content and addition of lactate makes lactated Ringer's solution an ideal replacement fluid with plasma like electrolyte composition (plasma contains bicarbonate but lactated Ringers solution has precursor of bicarbonate, the lactate). It supplies bicarbonate ions after hepatic metabolism of the lactate ions.

Lactated Ringer's solution is most versatile replacement fluid available. It may be used for volume expansion, replacement of fluid deficits and fluid delivery for reversal of all types of shock except cardiogenic shock. It is also useful in metabolic acidosis syndrome. Lactated Ringer's solution is often preferable when replenishing hypovolaemia in a dehydrated patient without major abnormalities. Once the patient is rehydrated, maintenance solutions are preferred for long-term fluid therapy. Although lactated Ringer solution is used also as a maintenance fluid, it is less preferred for long-term use because it contains more sodium and less potassium than a patient needs on a daily basis.

Dose

Dogs : 90 ml/kg, IV infusion for replacement.

Cats : 60 ml/kg, IV infusion for replacement.

Acetated Ringer's solution

Acetated Ringer solution is a replacement Ringer solution that contains sodium acetate, a precursor of bicarbonate. It differs from lactated Ringer solution because the sodium lactate is replaced by sodium acetate. Further metabolism of acetate ions to generate bicarbonate ions occurs in muscle and peripheral tissues instead of liver. This conversion of acetate to bicarbonate occurs more readily in shock because less oxygen is required for biotransformation. Similar to lactated Ringer's solution, acetated Ringer's solution is useful in metabolic acidosis syndrome.

3. Dextrose Solutions

Isotonic dextrose solution/Dextrose 5% in water (D5W)

Isotonic dextrose solution (Dextrose 5%, dextrose 5% in water, D5W) is 5 percent w/v dextrose in water. It is mainly used as a maintenance fluid and a source of free water.

Isotonic dextrose solution is usually considered as a source of energy, but actually it is more a source of free water because it produces only 170 kcal/L of energy, which is negligible in comparison with maintenance requirements of an animal. Once the dextrose is metabolised, free water is supplied to body. Oxidation of each gram of dextrose also supplies 0.6 ml of water that adds to total body volume. This solute free water is then distributed in proportions equal to the total body water i.e. two-thirds intracellularly and one-third extracellularly. Out of the one-third entering the ECF, about three quarters ends up in the interstitial space and one-quarter remains in the intravascular space. Therefore for any given volume of D5W, only 8 percent remains in the intravascular space, which is suitable for maintenance but not for replacement purposes.

Isotonic dextrose solution is used mainly in hypernatraemia and primary water depletion states where the dilutional effects lower the serum sodium levels. It is also used as a carbohydrate source to supplement concomitantly used polyionic solutions and as fluid supplement for patients with sodium intolerance. It is occasionally used as a carrier fluid for drugs.

D5W should not be used as replacement fluid because after the glucose is metabolised, the hypotonic/free water will rapidly leave the vascular space, thereby defeating the purpose of volume expansion. As D5W does not supply required electrolytes, it is also not used as the sole IV fluid in patients requiring maintenance therapy. Therefore, D5W is not suitable for volume resuscitation (e.g. shock), in intravascular volume maintenance or interstitial volume replacement.

Dose

Dogs & cats : 40 – 50 ml/kg, IV infusion, SC, or IP, as needed.

Hypertonic dextrose solutions

Hypertonic dextrose solutions (10%, 25% and 50%) are used mainly for the treatment of hypoglycaemia and ketosis. Hypertonic dextrose solutions should be used carefully because they may draw water from tissues into the plasma space, and water may be lost faster than glucose can be metabolised. Excessive use of glucose may also lead to glycosuria.

Dose

For hypoglycaemia and ketosis

Dogs & cats : 0.25 – 1 ml/kg, IV.
: 1 – 2 ml/kg, PO.
Cattle : 400 – 800 ml (total), slow IV or SC.
Sheep : 100 – 200 ml (total), slow IV or SC.

Hypotonic dextrose solution

Hypotonic dextrose is mainly used as 2.5 percent w/v dextrose in water. It is primarily used as a maintenance fluid either alone or mostly in combination with other fluids (half-normal saline or half-lactated Ringer's solution). When given at maintenance rates, 2.5% dextrose maintains the blood glucose at safe ranges without causing glycosuria.

Isotonic dextrose saline

Isotonic dextrose saline consists of 4.3% dextrose plus 0.18% sodium chloride. It supplies water, sodium and chloride and, therefore, is useful for the maintenance of fluid and electrolyte levels in small animals. The rapid metabolism of glucose supplies water in excess of solute, so it is useful for hypertonic dehydration also.

Dose

Dogs & cats : 40 ml/kg/day, IV infusion.

Other dextrose solutions

Some other less frequently used parenteral solutions of dextrose are also available commercially and are employed for specific purposes. These include hypertonic dextrose solutions of 20%, 30%, 40%, 60% and 70%. Several solutions of dextrose with sodium chloride are also used therapeutically and are available as 2.5% dextrose plus 0.45% sodium chloride (D2.5 & 0.45% NaCl), 5% dextrose plus 0.45% sodium chloride (D5 & 0.45% NaCl), 5% dextrose plus 0.9% sodium chloride (D5 & 0.9% NaCl), and 10% dextrose plus 0.9% sodium chloride (D10 & 0.9% NaCl). Combination of dextrose with other electrolytes such as 5% dextrose in Ringer solution, 2.5% dextrose in half-strength Lactated Ringer's solution and 5% in Lactated Ringer's solutions are also commercially available.

4. Sodium Bicarbonate Solutions

Sodium bicarbonate solutions (1.5%, 5% and 8.4%) are used in the treatment of severe metabolic acidosis. A 1.5% solution of sodium bicarbonate is approximately isotonic. Use of sodium bicarbonate solution is preferred in the emergency treatment of acidaemia because generation of bicarbonate through lactate metabolism generally takes few hours. In addition to its use in acidaemia, sodium bicarbonate is used to alkalinise the urine (see for detail Chapter 41). It is also used as adjunctive therapy in treating hypercalcaemic or hyperkalaemic crisis.

Bicarbonate should be used very carefully and must be administered slowly; otherwise as the body corrects its metabolic acidosis there may be an 'overshoot alkalosis'. Sodium bicarbonate should be used very carefully in animals with cardiac or renal failure because addition of sodium may increase the plasma osmolality and subsequently intravascular volume. Bicarbonate therapy can also cause changes in serum electrolyte, especially calcium and potassium under conditions of an alkaline pH e.g. protein binding of calcium is enhanced which may produce tetany. Similarly, correction of acidosis causes shift of potassium into cells that may produce hypokalaemia (therefore it is used in emergency treatment of hypercalcaemia and hyperkalaemia).

Dosages of sodium bicarbonate should be strictly followed and electrolyte and acid-base status should be frequently monitored. Sodium bicarbonate is reportedly incompatible with D5W and D5 lactated Ringer's solution, so it should not be mixed along with these parenteral fluids. The detailed pharmacology of sodium bicarbonate has been given in Chapter 41.

Dose

For acidosis

Dogs & cats : 0.5 – 1 mEq/kg, IV.
: 10 – 15 ml/kg, PO, 3 times daily.
Cattle & sheep : 2 – 5 mEq/kg, IV for a 4 to 8 hour period.

Notes:

a. One gram of sodium bicarbonate contains about 11.9 mEq each of sodium and bicarbonate; 84 mg of sodium bicarbonate contains 1 mEq each of sodium and bicarbonate.
b. 8.4% of sodium bicarbonate = 1 mEq/ml of sodium bicarbonate.

5. Potassium Solutions

Potassium chloride solution

Potassium chloride solution is available commercially in 2 mEq/ml and 3 mEq/ml concentrations. It is mainly used in the treatment of hypokalaemia. Potassium chloride injections should always be given by slow IV infusion not to exceed a rate of 0.5 mEq/kg/hour. Rapid injection may be cardiotoxic. Potassium solutions should not be used in renal failure, hyperkalaemia or atrioventricular block. Potassium chloride must be diluted with not less than 50 times its volume of sodium chloride 0.9% or other suitable diluents and then should be mixed well.

Dose

Dogs : up to 0.5 mEq/kg, IV infusion. The infusion rate should not exceed 0.5 mEq/kg/hour, and dose should not exceed 3 – 5 mEq K^+/kg, daily.
Dogs & cats : 1 – 3 mEq/kg, PO, once daily.

Notes

a. 1 mEq of KCl = 1 mMol of KCl = 74.5 mg of KCl.
b. 1 mEq of K^+ = 1 mMol of K^+ = 23 mg of K^+.

Potassium phosphate solution

Commercial injections of potassium phosphate are mixtures of the monobasic and dibasic salt forms. To avoid confusion, phosphorus concentration is prescribed in terms of millimoles of phosphorus. Commercial preparations of potassium phosphate usually provide 4.4 mEq potassium and 3 mMol phosphate per ml. Potassium phosphate solution is used to correct hypokalaemia or hypophosphataemia in diabetic ketoacidosis.

Potassium gluconate

Potassium gluconate is available in the form of oral powder and tablets, and is generally added in feed. Care should be taken not to produce hyperkalaemia, especially in patients with cardiac disease and renal impairment. Side effects of potassium gluconate may include gastrointestinal irritation, vomiting, diarrhoea and melaena.

Dose

Cats : 468 mg/4.5 kg, PO, 2 times daily with food.

6. Calcium Solutions

Calcium gluconate and Calcium borogluconate

Calcium gluconate and calcium borogluconate are the most widely used salts of calcium. Calcium gluconate is the calcium salt of gluconic acid, an oxidation product of glucose, whereas calcium borogluconate is a combination of five parts by weight of calcium gluconate and one part of boric acid. Calcium gluconate is sparingly soluble in water, but presence of boric acid in borogluconate increases solubility of calcium borogluconate (solubility 1: 5). Calcium gluconate for injection has a pH of 6-8.2 and is compatible with isotonic saline solution, lactated Ringer's solution, dextrose 5%, dextrose-lactated Ringer's solution and dextrose-saline combinations.

Calcium gluconate and calcium borogluconate are used for the prevention and treatment of hypocalcaemic conditions in small and large animals. Aqueous solutions of up to 20% are suitable for SC injection but stronger solutions are recommended for IV.

Calcium solutions should be used carefully to avoid hypercalcaemia, particularly in patients with cardiac or renal disease. Too rapid injection of calcium can cause hypotension, cardiac arrhythmias and cardiac arrest. Mild to moderate tissue reactions may occur after IM or SC administration of calcium and GI irritation and/or constipation after oral administration. Calcium solutions induced mild hypercalcaemia usually resolve with time, if renal functions are adequate, but more serious hypercalcaemias should be treated with IV normal saline and loop diuretics. Calcium solutions are mostly administered according to response of the patient, intensity of clinical signs, and blood analysis.

Dose

Calcium salts expressed as calcium

Dogs & cats : 75 – 500 mg of calcium, slow IV, according to patient's response.

Sheep : 0.5 – 1.5 g of calcium, SC or slow IV, according to patient's response.

Cattle & horses: 3 – 12 g of calcium, SC or slow IV, according to patient's response.

Calcium gluconate

Dogs & cats : 50 – 150 mg/kg, slow IV over 20 to 30 minutes (or IV infusion at rate not exceeding 0.5 – 1 ml/min).

Sheep, goats, cattle & horses : 150 – 250 mg/kg, slow IV (IP route may also be used).

Notes:

a. 11.2 mg calcium gluconate or 13.2 mg calcium borogluconate provides 1 mg of calcium.

b. Calcium gluconate 10% = calcium 8.9 mg/ml, calcium borogluconate 10% = calcium 15.2 mg/ml, and calcium chloride 10% = calcium 27.2 mg/ml.

Calcium chloride

Calcium chloride solution (10%) is occasionally used for the treatment of hypocalcaemia. Calcium chloride is less preferred for therapeutic purposes because it is more irritating than other parenteral salts and is more likely to cause hypotension. Calcium chloride releases three times as much as calcium ions as does calcium gluconate; therefore, any volume that is dosed in terms of calcium gluconate 10 percent should be reduced by one-third for calcium chloride 10 percent.

Other calcium salts

Several other parenteral and oral preparations of calcium are available commercially for the preventive and/or treatment of hypocalcaemia. These include calcium gluceptate (parenteral), calcium glycerophosphate (parenteral depot preparation) and calcium lactate, calcium sodium lactate and calcium hydrogen phosphate (all oral preparations). Calcium lactate and calcium sodium lactate are used for the treatment of rickets often in conjunction with vitamin D in small animals, whereas calcium hydrogen phosphate is used as calcium and phosphate supplement for the treatment of calcium and phosphorus deficiencies. These oral samlts are not of any value in acute hypocalcaemic conditions.

7. Magnesium Solutions

Magnesium sulphate

Magnesium sulphate solutions are available in 10, 12.5, 25 and 50 percent concentrations. Parenteral magnesium sulphate is used as a source of magnesium in magnesium deficient states including hypomagnesaemic tetany. One gram of magnesium sulphate hexahydrate supplies 8.1 mEq of magnesium. Intravenous administration of magnesium sulphate results in an immediate effect, whereas IM injection may require about 1 hour for effect. After administration, about 35% of magnesium binds to proteins and the reminder remains in free ionic form.

Magnesium sulphate should be used with caution in patients with impaired renal function and avoided in myocardial disease. Adverse effects related to magnesium salts are lagey as a result of overdosage and may include CNS depressant effects (drowsiness), cardiac depressant effects (bradycardia and hypotension), respiratory depression and muscular weakness. Very high magnesium levels may cause neuromuscular blocking activity and eventually cardiac arrest. Ventilatory support and administration of IV calcium may be required for severe hypermagnesaemia. Slow IV administration of magnesium sulphate added to calcium borogluconate reduces the possibility of seizures.

Dose

Magnesium sulphate 25%

Dogs & cats : 5–15 ml (total), IM or slow IV over 1 to 2 hours.

Sheep : 50–100 ml (total), SC, according to patients' need.

Cattle : 200 – 400 ml (total), SC, followed by a slow IV infusion of 500 ml of calcium-magnesium solution (calcium borogluconate 23% + magnesium chloride 6%).

Calcium-Magnesium solution (calcium borogluconate 25% + magnesium sulphate 10%)

Sheep : 50 – 100 ml (total), slow IV.

Cattle : 350 ml (total), slow IV.

8. Darrow's Solution

Darrow's solution, also called lactated potassium saline injection, consists of 0.4% sodium chloride + 0.27% potassium

Table 40.3: Composition of selected parenteral crystalloid solutions.

Solution	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{++} (mEq/L)	Cl^- (mEq/L)	Lactate (mEq/L)	Acetate (mEq/L)	Bicarbonate (mEq/L)	Dextrose (g/L)	Tonicity	Osmolarity (mOsm/L)	Calories (kcal/L)
0.9% Saline (NaCl)	154	–	–	154	–	–	–	–	Isotonic	308	–
3.0% Saline (NaCl)	513	–	–	513	–	–	–	–	Hypertonic	1026	–
7.5% Saline (NaCl)	1283	–	–	1283	–	–	–	–	Hypertonic	2566	–
0.45% Saline (NaCl)	77	–	–	77	–	–	–	–	Hypotonic	154	–
5% Dextrose (D5W)	–	–	–	–	–	–	–	50	Hypotonic/ Isotonic	278	170
10% Dextrose	–	–	–	–	–	–	–	100	Hypertonic	556	340
50% Dextrose	–	–	–	–	–	–	–	500	Hypertonic	2780	1700
2.5% Dextrose	–	–	–	–	–	–	–	25	Hypotonic	139	85
Ringer's solution	147	4	5	156	–	–	–	–	Isotonic	312	–
Lactated Ringer's solution	130	4	3	109	28	–	–	–	Isotonic	274	–
Acetated Ringer's solution	131	4	3	109	–	28	–	–	Isotonic	275	–
5% Sodium bicarbonate	595	–	–	–	–	–	595	–	Hypertonic	1190	–
8.4% sodium bicarbonate	1000	–	–	–	–	–	1000	–	Hypertonic	2000	–
2 mEq/ml Potassium chloride	–	2000	–	2000	–	–	–	–	Hypertonic	4000	–
3 mEq/ml Potassium chloride	–	3000	–	3000	–	–	–	–	Hypertonic	6000	–

Abbreviations: mEq = milliequivalent; L = litre; g = gram; ; mOsm = milliosmole; kcal = kilocalorie.

chloride + 0.58% sodium lactate. It is potassium rich and hence counters the depletion of ICF potassium seen in severe dehydration. The sodium lactate is metabolised to sodium bicarbonate and so has an alkalinising agent. Although the solution is intended for the treatment of metabolic acidosis such as that associated with diarrhoea and dehydration, it is not suitable for initial restoration of ECF volume in diarrhoea. Darrow's solution is now less used in veterinary therapeutics.

II. ORAL FLUID PREPARATIONS

The oral route is safe and economical means of fluid administration that allows both caloric and ionic requirements to be replaced and maintained. Oral fluid therapy is particularly useful in the treatment of dehydration associated with diarrhoea. The basic mechanism underlying the use of oral rehydration solutions is the intestinal co-transport of sodium and glucose leading to increased water uptake and reversal of dehydration. Therefore, sodium and glucose are present in most oral rehydration solutions. Fatty acid anions such as citrate, acetate or propionate may be included because they are metabolised to bicarbonate by the animal system, which assists in correction of acidosis. Potassium is usually present to counter K^+ loss. Cost and palatability also influence components contained in oral rehydrating solutions.

Commercially, a large variety of oral rehydration solutions are available. These solutions are mostly isotonic in nature because hypertonic solutions usually worsen diarrhoea due to osmosis or may produce hypernatraemia. The volume of fluid required and the frequency of administration generally depend on severity of condition. The oral rehydration solutions should be prepared/ reconstituted according to the manufacturer's instructions. These solutions may be taken voluntarily by the animal or are administered by a nasogastric tube. Hydration, serum electrolyte status and urine specific gravity should be monitored during oral rehydration. Water should be available a free choice.

GENERAL CONSIDERATION FOR FLUID THERAPY

The objectives of fluid therapy, whether parenteral or oral, include correction of extracellular fluid volume, plasma pH, blood glucose concentration and plasma concentration of specific ions. When fluid-therapy is under considerations, one should thoroughly evaluate the clinical condition and consider following points:

1. Type of fluid

Fluid therapy is indicated with aim of restoring the normal concentration of natural constituents of the ECF. Therefore, the state of hydration, electrolyte balance, acid-base balance, renal function and calorific balance of the animal should be considered before institution of fluid therapy. The choice of fluid generally depends on the type and extent of fluid losses incurred. Balanced crystalloids, also called replacement fluids (e.g. lactated Ringer's solution) are often preferred when replenishing hypovolaemia in a dehydrated patient without major electrolyte abnormalities. Once the patient is rehydrated, maintenance solutions are preferred for long-term fluid therapy. Markedly hypertonic crystalloid solutions (e.g. 7.5% saline solution) may be used in selected cases such as for certain types of shock. Table 40.4. presents some typical clinical conditions and parenteral solutions which may be appropriate.

2. Amount of fluid

The amount of fluid needed by a patient depends on the maintenance need, deficit and ongoing losses. The total 24 hours fluid requirement for dehydrated animal, therefore amounts to the sum of maintenance volume, dehydrated (deficit) volume and continuing loss (if any) volume.

a. *Maintenance volume:* The maintenance fluid volume is that amount of fluid which is normally required over a 24 hour period in a euhydrated patient. For practical purposes, taking the insensible (unknowingly, e.g. from

Table 40.4: Parenteral fluid therapy for various disorders.

Condition	Disturbance	Type of Fluid	Remarks
1. Haemorrhage	Blood loss, hypovolaemic shock.	Whole blood if PCV is low, plasma, lactated Ringer's solution; sodium chloride 7% followed by isotonic saline.	–
2. Heat stroke	Free water loss, hypernatraemic dehydration.	Sodium chloride 0.45% + dextrose 2.5%, followed by sodium chloride 0.9% or lactated Ringer' solution.	–
3. Burns, peritonitis, pancreatitis	Loss of plasma, water and electrolytes, hypovolaemic shock.	Plasma expanders, lactated Ringer's solution.	–
4. Vomiting	Loss of water, H^+, Na^+, Cl^-, K^+, metabolic alkalosis.	Ringer's solution, sodium chloride 0.9%.	Add potassium chloride (10-20 mEq/L), if therapy is longer than 3 days.
5. Diarrhoea	Loss of water, Na^+, HCO_3^-, Cl^-, (K^+ if long-term), metabolic acidosis.	Lactated Ringer's solution.	Add potassium chloride (10-20 mEq/L), if therapy is prolonged. Add bicarbonate (1-3 mEq/kg) if condition is severe and therapy is prolonged.
6. Anorexia	Depletion of calories and proteins.	Sodium chloride 0.18% + dextrose 4%, dextrose 5% (D5W).	–
7. Ketosis	Hypoglycaemia, accumulation of ketone bodies.	50% dextrose solution followed by 5% dextrose or glucose precursors by mouth.	–
8. Bowel obstruction	Loss of water, Na^+, HCO_3^-, Cl^-, metabolic acidosis.	Plasma expanders + lactated Ringer's solution.	Add bicarbonate (1-3 mEq/kg), if required.

respiration) and sensible (knowingly e.g. urine excretion) losses into consideration, the maintenance volume is about 60 ml/kg for an adult and about 130 ml/kg for a neonate of all species. Such replacement is required until the patient is able to take sustainable oral fluids.

b. *Deficit volume:* The deficit fluid volume is that amount of fluid which has already been lost by the animal during the disease. Fluid deficit must be estimated for most patients when they are initially examined. The deficit volume depends mainly on the degree or severity of dehydration, and is usually determined from the following formula

Deficit volume (ml) = Estimated percent dehydration (ml/kg) × body weight (kg) × 1000

The severity of fluid deficit or dehydration can also be roughly assessed from the physical parameters such as body weight, skin turgor, pulse rate and quality, and capillary refill time as given in Table 40.5. It is important to note that these parameters may not give true picture of fluid deficit in all conditions. For example, cachectic and very old animals lose their skin elasticity thereby showing a marked loss of skin turgor, even when they are not dehydrated. On the other hand, obese animals can retain normal resiliency even when they are moderately to severely dehydrate.

c. *Ongoing fluid losses:* This fluid volume includes loss of volume that is not normal and that is continuing during the treatment period. This includes loss of fluid due to continuation of vomiting, diarrhoea, polyuria, fever, tachypnoea, etc. Although the ongoing fluid loss depends on the individual clinical case, an estimate fluid loss in small animals through vomiting is 2 ml/kg body weight per vomit and through diarrhoea may be up to 200 ml/kg body weight daily. Replacement of continuing loses must be in addition to maintenance and deficit volume.

Exercise: A 2 year-old 20 kg dog is 8% dehydrated, and is vomiting 5 times daily while undergoing treatment. Calculate the total fluid requirement of the animal for first 24 hours.

Solution: The total fluid requirement of animal over the first 24 hours includes maintenance volume, deficit volume and ongoing fluid loss volume.

i. Maintenance volume = 60 ml/kg
= 60 × 20
= 1200 ml.

ii. Deficit volume = Estimated percent dehydration × body wt. (kg) × 1000
= 0.08 × 20 × 1000
= 1600 ml.

iii. Ongoing loss volume = 2 ml/kg/vomit
= 2 × 20 × 5
= 200 ml

Total fluid requirement = Maintenance volume + Deficit volume + Ongoing loss volume
= 1200 ml + 1600 ml + 200 ml
= 3000 ml or 3 litres.

3. Route of administration

Choice of route of fluid administration depends on the type of illness, severity and degree of dehydration, type of electrolyte

imbalance and condition of patient. Fluids may be administered by oral, subcutaneous, intraperitoneal, intravenous, rectal and intramedullary routes. Oral route is convenient and conducive for small animals and neonates, but is not suitable for conditions like unconsciousness, hypovolaemic shock and persistent vomiting. Whenever oral route is inadequate or not possible (e.g. vomiting, refusal to drink and unconsciousness), parenteral administration is preferred.

Intravenous route is the quickest way to replenish a deficit ECF and is the preferred route for severely dehydrated and hypovolaemic patients. This route also allows constant infusion, which can be advantageous, particularly to ill patients and when accurate delivery of fluid is necessary. Subcutaneous route is easier than IV administration and is considered adequate for correction of mild to moderate dehydration. However, one must not administer hypertonic (also dextrose solution of any tonicity) or irritating solution by SC route because it can draw fluids from the central compartment into the SC site or cause a large sore, respectively. Subcutaneous route is also not suitable in severely dehydrated animals or in patients with marked vasoconstriction because reduced peripheral perfusion in such animals allows poor absorption from injection site. In large animals, SC route is not much useful because it is difficult to administer large volume by this route. Intraperitoneal fluid administration is useful for dialysis and when IV and SC routes are not feasible or are contraindicated. However, hypertonic fluids should not be used by IP route because they may worsen the existing dehydration by drawing more fluid from the ECF. Intraperitoneal fluid administration is ineffective in the treatment of haemorrhagic shock. The rectal and intramedullary routes are not very effective and are not commonly used for fluid administration. The intramedullary route is reserved exclusively for resuscitation when vascular access is difficult to obtain as in neonate and exotic animals.

4. Rate of administration

The rate of fluid administration depends on the severity of the clinical condition. Initially in severely dehydrated cases, fluids may be given rapidly at rate up to 50 ml/kg/hour. In less severe cases, the infusion rate should be about 15-30 ml/kg/hour. High infusion rates (60-80 ml/kg/hour) should be used for resuscitation of animals in shock, only for a short period of time (20-30 minutes). In all cases, the rate of infusion should be slowed to about 5-15 ml/kg/hour after the first hour of administration.

Cardiopulmonary function should be monitored during infusion at high rates and restoration of urine output must be confirmed. Care should be taken to avoid over-infusion, especially in small animals. Hydration approaching normalcy is indicated when the production of urine begins and when the calculated deficit has been replaced; urine production over 1 ml/kg/hour may indicate over-infusion. On the other hand, if urine output is not established after 4 or more hours of fluid administration, rate should be slowed to 2 ml/kg/hour or less and every attempt should be made to establish renal function.

Table 40.5: Estimation of degree of dehydration.

Dehydration	Physical examination findings
< 4% (Insignificant)	Normal
4% (Very mild to mild dehydration)	History of vomiting or diarrhoea, but no significant gross abnormality. Skin slightly leathery, mucous membranes moist, no evidence of thirst.
6% (Mild to moderate dehydration)	Skin leathery, skin when lifted will peak but returns to normal slowly, oral mucous membranes dry but tongue is moist, hair coat is dull.
8% (Moderate to severe dehydration)	Loss of skin pliability and elasticity, skin when lifted will peak and stay, both oral mucous membranes and tongue are dry, slow capillary refill time.
10% (Severe dehydration)	All previous signs worse, extremities cold, eye sunken into orbits, weak and rapid pulse, difficult to stand, moderate to marked mental depression.
12% (Shock)	All previous signs worse, animal lying on the side and in shock.
> 12%	Animal is dying or is dead.

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CHAPTER 41 DRUGS AFFECTING URINARY pH AND TUBULAR TRANSPORT OF ORGANIC MOLECULES AND DRUGS

Drugs affecting urinary pH
- Urinary acidifiers
- Urinary alkalizers

Drugs affecting tubular transport of organic molecules and drugs

DRUGS AFFECTING URINARY pH

Kidneys control acid-base balance by excreting either acidic or basic urine. Unlike blood, the pH of urine can range from 4.5 to 8.0 depending on the acid-base status of the extracellular fluid. In veterinary medicine, normal pH of urine depends on the species as the urine pH of dogs and cats is acidic (5.5-7.0), while that of horses, cattle, sheep and goats is alkaline (7.0-8.0). As the alterations in urinary pH affects the degree of ionisation of electrolyte compounds (see Chapter 4), changes in urinary pH are generally implemented to either enhance efficacy/solubility of a drug in urine or facilitate excretion of a drug in urine. Changes in the urine pH are therapeutically achieved by use of either a urinary acidifier or a urinary alkalizer.

URINARY ACIDIFIERS

Urinary acidifiers (Urine acidifiers) are substances that cause acidification (decreases pH) of the urine. Urinary acidification enhances excretion of basic substances by increasing their ionisation in the filtrate that diminishes their passive reabsorption across the tubular wall. Acidification improves the antibacterial activity of hexamine, penicillins and tetracyclines in the treatment of urinary tract infections and may itself make conditions less conducive to bacterial growth. Urine acidifiers may also be used in animals with certain types of urinary or bladder stones in an attempt to dissolve the stones. Ammonium chloride, ascorbic acid, sodium acid phosphate, methionine, sodium chloride, ethylenediamine dihydrochloride and ammonium sulphate are clinically used to acidify the urine. Of these urinary acidifiers, ammonium chloride probably provides the most consistent changes in pH.

Ammonium chloride

Ammonium chloride (NH_4Cl) is an acidifying salt that occurs as colourless or white crystals having a saline taste. One gram of ammonium chloride contains 18.7 mEq of ammonium and chloride ions. Ammonium chloride is used primarily as a urinary acidifier and an expectorant. It is also used as a diuretic in forced acid diuresis. Its other applications in biology include as a nitrogen fertilizer in agriculture, a feed supplement for cattle and an ingredient in nutritive media for yeast microbiological organisms.

Mechanism of action

When used as a systemic acidifying agent, ammonium chloride in body is dissociated into the ammonium (NH_4^+) and chloride (Cl^-) ions. The dissociated NH_4^+ is converted by liver to urea with the release of a hydrogen (H^+) ion. This H^+ ion combines with a bicarbonate ion to form carbonic acid then to CO_2 and water. In the ECF, the chloride from the ammonium chloride replaces HCO_3^- leading to lower alkali reserves and development of hyperchloraemic acidosis. The net effects are decreased serum bicarbonate levels and reduced blood and urine pH.

Pharmacological effects

Ammonium chloride decreases the pH of blood and urine and has mild diuretic effect. The acidification effect of ammonium chloride in blood and urine occurs mainly due to its dissociation into ammonium and chloride ions. The diuretic effect is produced by the excess of chloride (released from ammonium chloride) in the renal tubular lumen, which takes sodium along with it to maintain electrical neutrality. However, the diuretic effect produced by ammonium chloride is mild and is compensated by kidneys usually after a few days of therapy. In the diuretic compensation process, the base-conserving mechanisms play important roles i.e. H^+ ions are secreted by tubules in exchange for Na^+, more of ammonia (NH_3) is generated by glutaminase system and excess of Cl^- is secreted in combination with NH_4^+. Ultimately the amount of NH_4Cl ingested is equal to the amount excreted by the kidneys and a refractory state is produced.

Side effects/Adverse effects

Development of metabolic acidosis is the most important adverse effect of ammonium chloride. Gastric irritation, nausea and vomiting may occur after oral administration of the drug. Overdosage of ammonium chloride may produce excessive thirst, hyperventilation, bradycardia and progressive CNS depression.

Treatment: Treatment of ammonium chloride intoxication includes correction of acidosis by IV administration of sodium bicarbonate or sodium acetate.

Contraindications and precautions

Ammonium chloride and other urine acidifiers are contraindicated in hepatic disease, uraemia, severe renal insufficiency and metabolic alkalosis secondary to vomiting. They are also contraindicated in patients with urate calculi or respiratory acidosis. Urine acidifiers should not be given to bitches those are pregnant or nursing. Urine acidifiers are preferably given with food to reduce the chances of GI side effects.

Clinical uses

In veterinary medicine, ammonium chloride is mainly used as a urinary acidifying agent to help prevent and dissolve certain types of uroliths, to enhance renal excretion of drugs and toxicants (e.g. quinidine) and to enhance efficacy of certain antimicrobial drugs (e.g. tetracycline and nitrofurantoin). It has also been used intravenously for the rapid correction of metabolic alkalosis. Its use as diuretic has declined mainly due to its limited and short-term effect and availability of better drugs.

Dose

For urine acidification

Dogs : 200 mg/kg/day, PO, divided into 3 doses.
Cats : 20 mg/kg, PO, 2 times daily.
Cattle : 15 g (total), PO.
Sheep & swine : 1-2 g (total), PO.

Ascorbic acid

Ascorbic acid (Vitamin C) is a water-soluble vitamin that is involved mainly in tissue repair, collagen formation and some oxidation-reduction reactions. Ascorbic acid may be used as a urinary acidifier, but it is inconsistent in lowering urinary pH. The pharmacology of ascorbic acid is given in detail in Chapter 55.

Dose

For urine acidification

Dogs : 100 – 500 mg (total), PO, 3 times daily.
Cats : 100 mg (total), PO, 3 times daily.

Sodium acid phosphate

Sodium acid phosphate (Sodium dihydrogen phosphate, sodium biphosphate) is chemical compound of sodium with a phosphate counter ion. Sodium dihydrogen phosphate occurs as colourless to white crystals or as a white crystalline powder; sodium dihydrogen phosphate (anhydrous) occurs as white powder or granules. Sodium acid phosphate is occasionally used as an acidifier of urine. It is useful mainly in cats and dogs. Sodium acid phosphate is useful in cystitis and other urinary tract infections in which urine becomes alkaline.

Dose

For urine acidification

Dogs : 150 – 300 mg (total), PO, 3 times daily.

Methionine

Methionine (dl-Methionine) is a sulphur containing amino acid, which is used occasionally as a urine acidifier in domestic animals. In small animals, methionine is used mainly in the treatment and prevention of certain types of stone formation (e.g. struvite) and to reduce ammoniacal urine odour. However, methionine should not be given to animals with pre-existing acidosis or urate calculi. Methionine may cause Heinz-body haemolytic anaemia in cats and is more toxic to kittens. Allergic reactions to methionine may also occur and may include swelling of the face, seizures, pale gums or coma.

Dose

For urine acidification

Dogs : 0.2 – 1 g (total), PO, 3 times daily.
Cats : 0.2 – 1 g (total), PO, once daily.
Cattle : 20 – 30 g (total), PO.

URINARY ALKALIZERS

Urinary alkalizers or Urinary alkalinizers are substances which cause alkalinisation (increase pH) of the urine. Alkalinisation of urine increases the antibacterial activity of some antimicrobial drugs like aminoglycosides, erythromycin and fluoroquinolones. The urinary alkalizers are also of value during the administration of sulphonamides because they increase solubility of sulphonamides and thus decrease danger of crystalluria and damage to the kidneys. Alkalinisation of urine also decreases formation of uric acid and cystine stones. Sodium bicarbonate and potassium citrate are commonly used urine alkalizers.

Sodium bicarbonate

Sodium bicarbonate (Sodium hydrogen carbonate, baking soda, $NaHCO_3$) is a white crystalline powder having a slightly saline or alkaline taste. It is soluble in water and insoluble in alcohol. Sodium bicarbonate is used mainly as an alkalizer in the treatment of metabolic acidosis and as an antacid in treatment of acid indigestion.

Mechanism of action

After oral or parenteral administration, sodium bicarbonate dissociates into sodium (Na^+) and bicarbonate (HCO_3^-) ions. Bicarbonate ion is the conjugate base component of bicarbonate: carbonic acid buffer system. The increase in serum bicarbonate concentration causes rise in the normal 20:1 HCO_3^-: H_2CO_3 ratio and thus an elevation of the blood and urine pH

Pharmacological effects

The pharmacological effects of sodium bicarbonate are mainly due to its alkalinisation effects. When taken orally or given IV, it causes rise in the pH of blood and urine and may cause metabolic alkalosis. The alkalinisation of urine helps in the excretion of certain drugs (e.g. salicylates) by increasing their ionisation and decreasing their tubular reabsorption. In hyperacidity, it effectively neutralizes gastric acid (see for detail Chapter 42). Release of carbon dioxide in stomach, however, may cause a rebound acid secretion.

Side effects/Adverse effects

Sodium bicarbonate therapy may lead to metabolic alkalosis, hypokalaemia, hyponatraemia, volume overload and congestive heart failure. The adverse effects are prominent if sodium bicarbonate is used in high doses by parenteral route. Very high doses of sodium bicarbonate may cause severe alkalosis (see also Chapter 42).

Treatment: Treatment of severe alkalosis induced by sodium bicarbonate may require intravenous calcium therapy. Mild alkalosis may be treated by simply discontinuing the sodium bicarbonate therapy.

Contraindications and precautions

Sodium bicarbonate therapy is contraindicated in patients with metabolic or respiratory alkalosis. It should not be used in cases of hypocalcaemia or hypochloraemia. Sodium bicarbonate should be cautiously used in patients with congestive heart failure, hypertension, volume overload and nephrotic syndrome because it enhances sodium load in the body.

Drug interactions

Sodium bicarbonate shows interactions with a number of drugs mainly by altering their urinary excretion. In the alkaline urine, excretion of weakly acidic drug (e.g. salicylates) is increased,

/hile excretion of weakly basic drugs (e.g. amfetamine and uinidine) is decreased. The solubility of fluoroquinolones (e.g. iprofloxacin and enrofloxacin) is usually decreased in an alkaline H. Use of sodium bicarbonate concurrently with diuretics (e.g. ɔop diuretics) may cause hypochloraemic alkalosis. Oral dministration of sodium bicarbonate can also alter rate and/or xtent of absorption of many orally administered drugs (see for etail Chapter 42).

Clinical uses

Sodium bicarbonate is used mainly in the treatment of ıetabolic acidosis and to alkalinise the urine. Alkalinization of rine is utilized for the treatment of aspirin overdose and uric acid enal stones. Sodium bicarbonate also acts as antacid and xpectorant, but is less used for these actions nowadays due to nore adverse effects and availability of better drugs. It is ıccasionally used as adjunctive therapy in treating hypercalcaemic ır hyperkalaemic crisis. It can also be applied topically as a paste, vith three parts sodium bicarbonate to one part water, to relieve nsect bites.

Dose

For urine alkalinisation

Dogs & cats : 10 – 50 mg/kg, PO, 2 to 3 times daily. Dose may be adjusted to achieve the desired urinary pH.

Potassium citrate

Potassium citrate is a potassium salt of citric acid with the molecular formula $C_6H_5K_3O_7$. It occurs as white crystalline powder or granular crystals. Potassium citrate is soluble in water and has alkaline taste. Potassium citrate is rapidly absorbed when given by mouth and is excreted in the urine as the carbonate. Potassium citrate is used mainly as urine alkalizer for treatment of urinary tract infections. It is considered effective in reducing the pain and frequency of urination when these are caused by highly acidic urine. Potassium citrate therapy is useful in the management of calcium oxalate and urate urolithiasis because citrate complexes with calcium to form salts that are more soluble than oxalate salts. Potassium citrate is also an effective way to treat/manage gout and arrhythmia, if the patient is hypokalaemic. Potassium citrate should not be used in patients with renal or cardiac impairment. Dosing of potassium citrate may be guided by measuring urinary pH, which should be monitored at or above 7.0 in small animals.

Dose

For urine alkalinisation

Dogs & cats : 50 – 75 mg/kg, PO, 2 times daily.

Sodium citrate

Sodium citrate is the sodium salt of citric acid. It occurs as white crystalline powder or granular crystals having a saline taste. It is soluble in water and slightly deliquescent in moist air. Sodium citrate is used primarily as an anticoagulant (see Chapter 35). Occasionally, it is used as an alkalizer of blood and urine, and as a diuretic agent.

Dose

For urine alkalinisation

Dogs : 0.3 – 1.2 g (total), PO.

Cattle & horses: 4 – 15 g (total), PO.

DRUGS AFFECTING TUBULAR TRANSPORT OF ORGANIC MOLECULES AND DRUGS

Some drugs alter transport of organic molecules and drugs across the renal tubular epithelial barriers. In human medicine, these drugs are used mainly to increase the rate of excretion of uric acid and are often called uricosuric agents. In veterinary medicine (also human medicine), these agents are used occasionally to reduce the renal tubular excretion of penicillins and cephalosporins.

Probenecid

Probenecid is a highly lipid-soluble organic acid that was developed in 1951 to inhibit the renal tubular secretion of penicillin so that its duration of action could be prolonged. It has opposite effect on uric acid i.e. it promotes excretion of uric acid and reduces its blood levels.

Mechanism of action

Probenecid acts by competitively blocking an active organic anion transporter (OAT) present in renal tubules, which normally reclaims uric acid from the urine and returns it to the plasma. Blockade of OAT also interferes with either excretion or reabsorption of various other organic molecules and drugs operating through the same system. Drugs that are predominantly secreted by renal tubules (e.g. penicillin) show sustained blood levels as they are not easily excreted in the urine. On the other hand, drugs/organic molecules (e.g. uric acid) which are largely reabsorbed by tubular active transport are rapidly excreted in urine. As the transport is bi-directional, the net effect of tubular transport blockade depends on whether excretion or reabsorption of that particular organic molecule is more important.

Pharmacological effects

Probenecid does not have any significant pharmacological effect of its own except that produced by blockade of active renal tubular transport.

Pharmacokinetics

Pharmacokinetic data on probenecid in domestic animals are scarce. In humans, probenecid is completely absorbed from the GI tract after oral administration, with peak concentration seen in 3 hours. About 85-95% of drug is bound to plasma proteins. The remaining free drug is actively secreted by proximal convoluted tubule with a small unbound drug gaining access to glomerular filtrate. The half-life of probenecid in plasma is dose-dependent and varies from 5 to 8 hours.

Side effects/Adverse effects

Probenecid is reported to be well tolerated in humans with occasional GI irritation and hypersensitivity reactions (e.g. skin rashes). Very high doses may produce CNS stimulation, convulsions and death from respiratory failure. Side/adverse effects of probenecid in animals are not documented.

Drug interactions

Probenecid inhibits urinary excretion of penicillins, cephalosporins, sulphonamides, methotrexate and indometacin, thereby sustaining their plasma concentration and prolonging their duration of action. It inhibits tubular secretion of nitrofurantoin, which may not attain its antibacterial action in urine. Probenecid also blocks response to furosemide due to competition for active tubular secretion. Probenecid inhibits biliary excretion of rifampicin. Salicylates block uricosuric action of probenecid.

Clinical uses

In human medicine, probenecid is used mainly as a uricosuric agent in the treatment of gout. Probenecid may also be used as adjunct in penicillin therapy, where it increases penicillin concentration in plasma about 2 fold or more. This is particularly useful when penicillin is required in high concentrations in resistant infection. Here probenecid reduces the dose, but prolongs the duration of action of penicillin. In veterinary practice, probenecid may be given with penicillins (e.g. amoxicillin) and select cephalosporins to exotic birds to allow alternate dosage and, therefore, reduce stress from handling.

Dose

Exotic birds (> 200 g body wt.) = 200 mg/kg on alternate days in conjunction with penicillins (e.g. amoxicillin).

Sulfinpyrazone

Sulfinpyrazone (Sulphinpyrazone) is a pyrazolone derivative and is related structurally to phenylbutazone. It is a strong acid (pK_a 2.8), which readily forms soluble salts. Similar to probenecid, sulfinpyrazone inhibits tubular reabsorption of uric acid and reduces renal tubular secretion of some drugs. It is mainly used as uricosuric agent in human medicine in the treatment of chronic gout. It is not used to control acute attacks, and may in fact exacerbate symptoms if started during an attack. Sulfinpyrazone may be used in conjunction with allopurinol. Important side/adverse effects may include gastrointestinal disturbances and occasionally rashes and salt and water retention. Rarely, blood disorders and gastrointestinal ulcers and bleeding may occur. Sulfinpyrazone should not be recommended in patients with peptic ulcers or kidney disease.

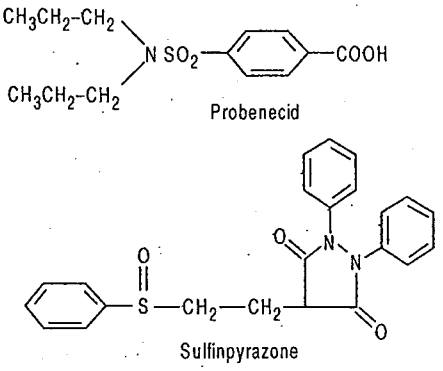


Fig. 41.1. Chemical structures of probenecid and sulphinpyrazone.

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SECTION-VII

DRUGS ACTING ON DIGESTIVE SYSTEM

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CHAPTER 42 DRUGS AFFECTING GASTROINTESTINAL SECRETIONS

Drugs affecting salivary secretion
- Salvary stimulants/Sialics
  - Classification
  - Indirect acting sialics
  - Direct acting sialics
- Salivary inhibitors/Antisialics

Drugs affecting gastric secretion
- Physiology
- Secretion of gastric acid
- Regulation of gastric acid secretion
- Mucus defences
- Drugs stimulating gastric secretion
  - Classification
  - Gastrin analogues
  - Histamine and analogues
  - Stomachics
- Drugs inhibiting gastric secretion
  - Classification
  - H_2-receptor antagonists
    - Structure
    - Mechanism of action
  - Proton pump inhibitors
  - Muscarinic receptors antagonists
    - Non-selective muscarinic receptor antagonists
    - Selective M_1-receptor antagonists
  - Prostaglandin analogues

Acid neutralsing and cytoprotective drugs
- Acid neutralising drugs /Antacids
  - Mechanism of action
  - Classification
  - Systemic antacids
  - Non-systemic antacids
    - Mechanism of action and pharmacological effects
    - Drug interactions
    - Clinical uses
    - Aluminium salts
    - Magnesium salts
    - Calcium salts
    - Antacid combinations
- Cytoprotective drugs

The digestive system is made up of the gastro-intestinal tract and associated organs which help in the digestion and absorption of food. Various physiological processes involved in digestion of food include motility of gastrointestinal tract, secretions of glandular and epithelial cells, enzymatic hydrolysis of macro- and micro-molecules, absorption of end products, and elimination of the residue. In the entire process of digestion, gastro-intestinal secretions play very important roles. In the mouth, chewing and mastication is facilitated by saliva secreted from the salivary glands. When the food travels down the oesophagus into the stomach, the gastric secretions containing hydrochloric acid and enzymes cause mechanical break down and chemical alteration of food. In the small intestine, the food is properly digested and largely absorbed. Finally through the large intestine the undigested food is eliminated during defecation. Various disease processes of digestive system and associated organs may interfere with one or more of the digestive processes mentioned above affecting the digestion and thus health of animals. Depending on the condition, various therapeutic agents are used to modify or influence one or more types of physiological process including the gastrointestinal secretions and bile flow involved in digestion of food.

DRUGS AFFECTING SALIVARY SECRETION

Saliva is the watery and usually frothy substance produced in the mouths of humans and most other animals. Saliva is continuously secreted into the oral cavity from the salivary glands and is composed of water, electrolytes, mucus and various enzymes. The digestive functions of saliva include moistening food and helping to create a food bolus, so it can be swallowed easily. Saliva contains the enzyme amylase that breaks up starch into sugar and also salivary lipase to initiate fat digestion. Regulation of salivary secretion is largely through the salivary nuclei located in brain stem, which are excited by taste and tactile stimuli from tongue and other areas of the mouth. Higher centres in the CNS also have excitatory and inhibitory input in the salivary nuclei, thus increasing or decreasing flow of saliva. Salivary secretions consists of two components, one being a watery secretion governed mainly by the parasympathetic nervous system and other more viscous governed by sympathetic nervous system.

SALIVARY STIMULANTS/ SIALICS

Salivary stimulants (Sialics, sialogogues, sialagogues) are agents which increase the volume and fluidity of saliva. By increasing the volume and fluidity of saliva, they stimulate appetite

and even digestibility of food. They are infrequently used clinically to treat hypoptyalism (decreased salivary flow) or xerostomia, but some agents (e.g. bitters) which stimulate taste buds and thus salivary flow are commonly incorporated in 'tonic' preparations.

Classification

On the basis of mode of action, sialics are categorized into two types – indirect acting sialics and direct acting sialics.

I. Indirect acting sialics
   e.g. gentian, nux vomica and quassia.
II. Direct acting sialics
   e.g. carbachol and arecoline.

I. Indirect Acting Sialics

Indirect acting (Reflex acting) sialics are mainly vegetable drugs, which stimulate taste buds on tongue due to their mild irritant action to produce increased salivation. These drugs possess bitter taste and mild irritant action; therefore they are commonly called bitters. Bitters take some time to produce their effect, so they are usually given 15-30 minutes before meal. The long-term use (>7 days) of bitters should be avoided as their response decreases with time and they can also produce gastric catarrh. Although regarded as obsolete, bitters are still included in many remedies to stimulate salivation and appetite.

Gentian

Gentian is the dried rhizome and root of a genus of flowering plants *Gentiana.* Gentian obtained from *Gentiana lutea* (Great Yellow Gentian), a large perennial herb native to the mountains of central and southern Europe, is widely employed in medicine, though the roots of several other species are said to be equally efficacious. Gentian has a characteristic odour and initially a sweet taste followed by bitterness. Gentian contains the bitter glycosides amarogentin and gentiopicrin (~2%) as the principal active constituents. The former is one of the most bitter natural compounds known and is used as a scientific basis for measuring bitterness. Other bitter constituents are gentisin, gentiopicroside and gentiamarin.

Bitter is used as a bitter tonic and stomachic for increasing appetite in humans and animals. It also enhances the purgative action of aloes. It may also be used for stimulating the liver and gall bladder functioning and treating all cases of debility. It should not be prescribed for patients with gastric or duodenal ulcers.

Dose

Gentian powder

Dogs	:	0.3 – 1.2 g (total), PO.
Cattle & horses	:	30 – 60 g (total), PO.
Sheep & pigs	:	4 – 8 g (total), PO.

Nux vomica

Nux vomica is the dried and ripe seeds of *Strychnos nux-vomica,* a deciduous tree native to India. The major alkaloids of therapeutic importance in nux vomica are strychnine (1.25%) and brucine (1.5%), which are derived from the seeds. The nux vomica powder is used to alleviate indigestion and as a stimulant on the gastro-intestinal tract. In the mouth it acts as a bitter sialogogue, increasing appetite by stimulating salivary secretion. In the stomach, it stimulates gastric muscles and increases flow of gastric juices. It also enhances peristalsis and is occasionally combined with cascara and other laxatives to treat chronic constipation. After absorption, strychnine may reach systemic circulation in sufficient concentration and cause stimulatory action on CNS, heart and respiratory system. Strychnine is a potent poison, so preparation containing nux vomica should be used cautiously and only in recommended doses.

Dose

Nux vomica powder (Powder is adjusted to contain 1.2% of strychnine)

Cattle	:	4 – 8 g (total), PO.
Horses	:	1.3 – 4 g (total), PO.

Quassia

Quassia is generally obtained from the dried stem wood of *Picraena excelsa,* which is commercially known as Jamaica quassia. The quassia powder is yellowish white in colour, odourless with strong bitter taste. Quassia contains the amaroid (terpenoid) compound known as quassin. Quassia is used as a bitter tonic and its action resemble gentian. In small doses quassia increases the appetite, but in large doses it acts as an irritant and causes vomiting. It has some anthelmintic, parasiticide and fly repellent properties also.

II. Direct Acting Sialics

Direct acting sialics directly stimulate the salivary gland to produce increased flow of saliva. Cholinergic sialics like carbachol and arecoline stimulate the salivary gland directly through muscarinic receptors and lead to copious flow of serous saliva. Alpha-adrenoceptor agonists promote the secretion of scanty amounts of viscous saliva. Ptyalism is seen in cases of mercurialism and intoxication by organophosphorus, carbamate and pyrethroid insecticides. As such, direct acting sialics have no/little therapeutic use.

SALIVARY INHIBITORS/ANTISIALICS

Salivary inhibitors (Salivary suppressants, antisialics, antisialogogues) decrease the flow and fluidity of saliva, often accompanied by decrease in respiratory and digestive secretions. Therapeutically, antisialics are often required during surgery of mouth or salivary glands and administration of general inhalant anaesthetics. Disorders affecting the CNS, oral lesions, and pharyngeal and oesophageal diseases may also stimulate salivary secretion and may require use of antisialics. Inhibition of salivation is also seen as a side effect of several drugs including anticholinergics (e.g. atropine), antihistamines (e.g. diphenhydramine) and neuroleptics (e.g. thioridazine).

Therapeutically, antimuscarinic drugs like atropine, hyoscine and glycopyrronium are mainly used as antisialics.

Atropine and Hyoscine

Atropine and hyoscine are naturally occurring antimuscarinic drugs which have been used as pre-anaesthetics, primarily to reduce salivary and bronchial secretions and to treat vagally mediated cardiac arrhythmias caused by the procedure or anaesthetic drugs. Their routine use in anaesthesia is declining because newer halogenated inhalation anaesthetics cause less irritation to the airways than the ether. However, these drugs may be indicated in cats and small dogs in which a small amount of saliva and respiratory secretion may block the airway. Atropine and hyoscine are normally not used as pre-anaesthetics in horses because the central excitation, mydriasis and decreased

gastrointestinal motility produced by them are not desirable. Administration of antimuscarinic drugs to ruminants is also not practiced routinely as they do not inhibit salivation effectively, but may result in more viscid (sympathetic) secretion than can be difficult to remove from the mouth and pharynx. Hyoscine has greater central effects, which are generally undesirable. The pharmacology and dosages of atropine and hyoscine are given in Chapter 14.

Glycopyrronium

Glycopyrronium (Glycopyrrolate) is a longer acting synthetic quaternary atropine substitute. It is a potent antisecretory and antibradycardiac drug, which acts rapidly and is less likely to produce central effects. Glycopyrronium is used exclusively for pre-anaesthetic medication and is approved for use in dogs and cats. Unlike atropine, glycopyrronium may be better for use in horses, if required, because it does not cross blood-brain barrier and so does not produce central effects. It also causes reduced intensity of tachycardia compared to atropine. Glycopyrronium is preferred in caesarean section because it does not cross the placenta. For maximum effect, glycopyrronium should be administered about 15 minutes before general anaesthetic administration. Adverse effects of glycopyrronium mainly include mydriasis, tachycardia and xerostomia in dogs and cats.

Dose

Dogs	:	0.01 – 0.02 mg/kg, SC, IM, or IV.
Cats	:	0.011 mg/kg, IM.
Horses	:	0.001 – 0.003 mg/kg, IV.

DRUGS AFFECTING GASTRIC SECRETION

The stomach secretes about 2.5 litres of gastric juice daily. The principal secretions of stomach include pepsinogens from the chief or peptic cells and hydrochloric acid and intrinsic factor from the parietal or oxyntic cells. In addition, mucus is secreted by mucus secreting cells.

PHYSIOLOGY

Most domestic animals have only glandular mucosa (horses have also a non-glandular mucosa), which is divided into three regions: cardiac mucosa, parietal mucosa and pyloric mucosa. These areas contain gastric glands of similar structure, but with different types of secretions. The glandular mucosa of stomach has frequent invaginations or pores called gastric pits. Base of each pit continues into the opening of one or more gastric glands. The major surface cells of the stomach as well as lining of the pit are covered with surface mucous cells, which provide protection to the stomach.

The glands in the cardiac mucosa (cardiac glands) secrete only mucus that is alkaline and probably protects adjacent oesophageal mucosa from the acid secretions of stomach. Within parietal area, the glands contain three types of cells i.e. parietal cells, mucus neck cells and chief cells (Figure 42.1). Parietal cells are clustered in the neck or proximal part of the gland and secrete hydrochloric acid. Mucous neck cells are distributed among the parietal cell in the neck of glands and secrete a thin mucous which is less viscous than that of the surface mucous cells. The chief cells are located at base of the gastric gland and secrete pepsinogen, the precursor to the digestive enzyme pepsin. The gastric glands in the pyloric mucosa (pyloric gland) have no parietal cells, but contain the gastrin producing G cells. The pyloric cells also secrete pepsinogen.

Secretion of Gastric Acid

Gastric acid is a digestive fluid, formed in the stomach. Gastric acid (HCl) secretion at cellular level involves generation and subsequent secretion of hydrogen ions by the parietal cells of the gastric mucosa. The secretion of parietal cell is an isotonic solution of HCl having a pH less than 1.0. The regulation of acid secretion by parietal cells is especially important in peptic ulcers and hyperacidity and constitutes a particular target for drug action.

The pH of gastric acid is 1 to 2 in the stomach lumen, the acidity being maintained by the proton pump (hydrogen ion pump). The proton pump that is located at the apical (luminal) membrane of parietal cells and associated with smooth endoplasmic reticulum is a hydrogen potassium adenosine triphosphatase (H^+-K^+-ATPase). This enzyme exchanges hydrogen ion for potassium ions pumping one K^+ into the cell for each H^+ secreted into the lumen, utilising one molecule of adenosine triphosphate (ATP). The K^+ that accumulates within the cell is released back into the lumen in combination with Cl^- (a symport transport). This allows recycling of potassium ions, resulting in the net secretion of hydrogen and chloride, with little movement of potassium (Figure 42.1). Chloride and hydrogen ions are thus secreted separately from the cytoplasm of parietal cells and mixed in the canaliculi. Gastric acid is then secreted into the lumen of the oxyntic gland and gradually reaches the main stomach lumen.

Hydrogen ions for secretion come from the dissociation of intracellular carbonic acid (H_2CO_3) in the cell. Carbonic acid originates from water and carbon dioxide through the action of carbonic anhydrase, an enzyme found in high concentration in the gastric mucosa. Intracellular HCO_3^- anion is exchanged for chloride anions at the cell's non-luminal surface. The bicarbonate is then secreted into the blood. During the periods of intense secretion by the gastric glands, large amounts of bicarbonate are released into the blood resulting in alkalinisation of blood. Normally, this is reversed when bicarbonate enters the intestine and utilised for neutralisation of gastric secretion resulting in only small and transient changes in blood pH. However, in some disease states when secretions of stomach are prevented from entering the intestine or are lost from body because of vomiting, the pH of blood can rise dangerously to produce alkalosis.

Regulation of Gastric Acid Secretion

Gastric acid production is regulated by the autonomic nervous system and some hormones. The proton pumps located at the apical membrane of parietal cells are activated by acetylcholine (via vagus nerve), histamine and gastrin acting via their own receptors. Out of these physiological secretogogues, histamine acting through H_2 receptors plays the dominant role because the other two gastrin and acetylcholine act partly directly and partly indirectly by releasing histamine from the paracrine enterochromaffin like (ECL) cells called 'Histaminocytes' located in the oxyntic glands (Figure 42.1). As such, histamine participates in acid response to gastrin and acetylcholine at more than one level and H_2-receptor blockers suppress not only histamine but also acetylcholine, pentagastrin, and other secretory stimuli. Of various pathways, acetylcholine pathway appears to be less important in small animals and vagal effects are expressed largely indirectly through histamine and gastrin.

Intracellular messengers mediating gastric acid secretions vary according to the stimulated receptor. Histamine increases cAMP production, which subsequently stimulates cAMP-dependent protein kinases. Gastrin and muscarinic stimulation increases cytosolic calcium, probably by increased influx through selective receptor activated calcium channels in the cell membrane. Prostaglandins of E series (PGE_2) produced by gastric mucosa inhibit acid secretion by opposing cAMP production (in parietal cells) and gastrin release (from G cells). Vasoactive intestinal peptide, cholecystokinin, and secretin all inhibit production.

Gastric acid secretion occurs in four phases viz. cephalic, gastric, intestinal and basal. In cephalic phase, anticipation of food causes release of acetylcholine by the gastrointestinal intrinsic nervous system into the vicinity of G cells and parietal cells, which respond by secreting gastrin and HCl, respectively. Gastrin through circulation finds its way to the parietal cells and increases further HCl secretion. Food entering stomach initiates the second phase or 'gastric phase' of gastric secretion. Distension of stomach by food stimulates stretch receptors causing direct nervous (acetylcholine) stimulation of the G and parietal cells. In addition, food raises gastric pH, that removes the inhibitory effect of acid on gastric G cells (when stomach pH falls to 2, gastric secretion declines; at a pH of 1, it is completely abolished to begin only when the gastric pH approximates 3.0 to 3.5), further stimulating production of gastrin. As acid contents from the stomach enter the duodenum, an intestinal negative feedback mechanism decreases further the gastric acid production. Rising of intestinal pH in turn stimulates gastric acid production, the third phase of gastric acid secretion. Fourth phase of gastric secretion is basal phase and occurs in the absence of external stimuli. The amount of basal secretion varies among animals and is not well characterised in dogs and cats. In humans, basal secretion follows a circadian rhythm, reaching a peak at midnight and a lowest at 7.00 AM.

Mucus Defences

Local secretion of prostaglandin E_2 (PGE_2) is an important defence mechanism that acts to prevent or repair damage to the GI tract. Endogenous prostaglandins of E series modulate (decrease) hydrochloric acid secretion from gastric parietal cells, increase secretion of mucus and bicarbonate by adjacent superficial epithelial cells, and enhance mucosal blood flow and epithelialisation. Enhanced secretion of bicarbonate neutralises the hydrochloric acid in lumen and secretion of thick alkaline

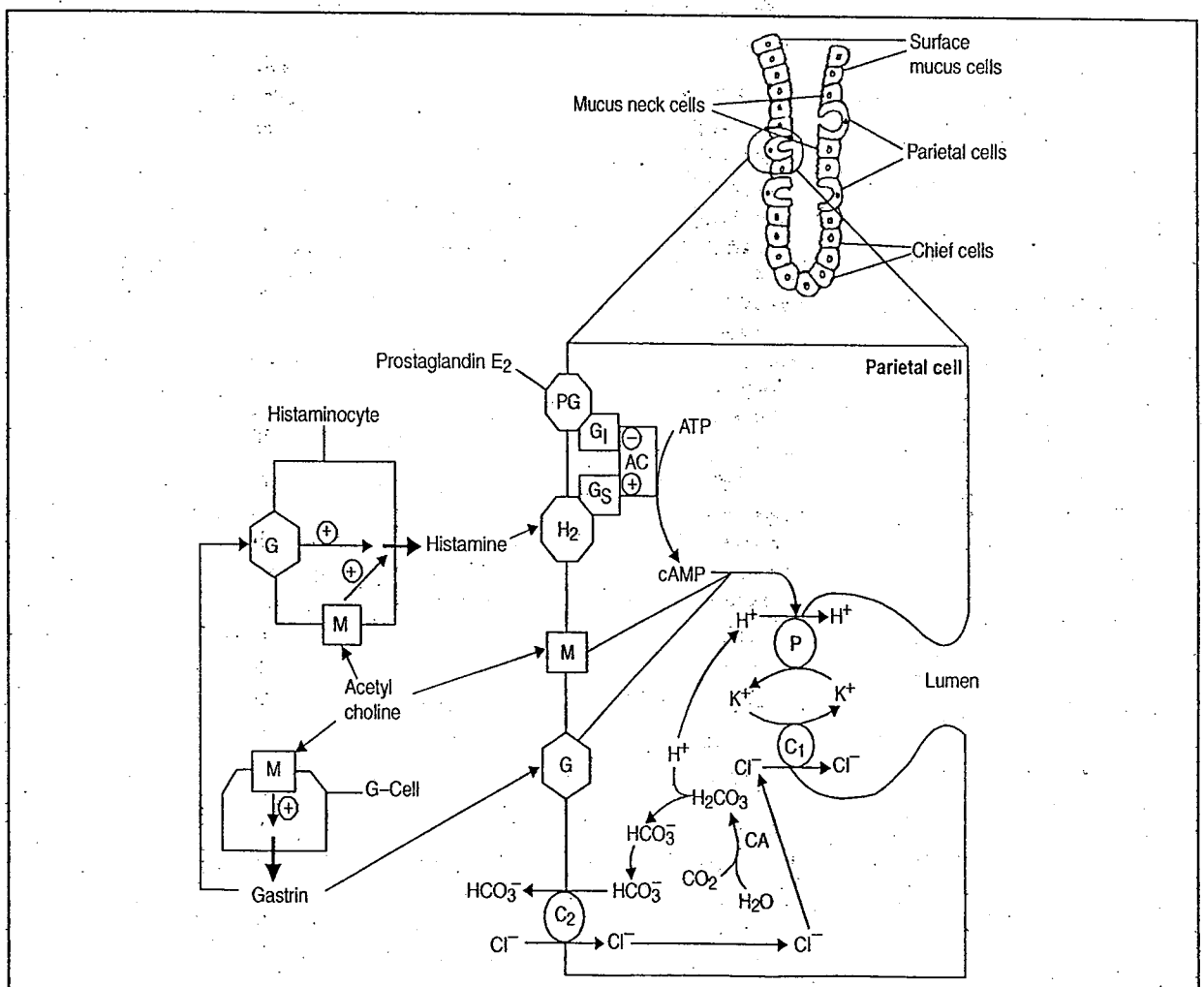


Fig. 42.1. Schematic representation of secretion of gastric acid by parietal cell and its regulation. Anatomical illustration of gastric gland with various secretory cells is shown above (see text for detail).

Abbreviations : AC = adenylate cyclase ; ATP = adenosine triphosphate ; cAMP = 3' 5' - cyclic adenosine monophosphate ; G = gastrin receptor ; M = muscarinic receptor; H_2 = H_2 - histamine receptor ; PG = prostaglandin receptor ; G_i = inhibitory G-protein ; G_s = stimulatory G-protein ; CA = carbonic anhydrase ; P = proton pump; C_1 = $K^+ - Cl^-$ symporter ; C_2 = HCO_3^- - Cl^- antiporter ; + = activation/stimulation; – = inhibition.

mucus traps and neutralises inward moving hydrogen ions. A gastric epithelial barrier composed of active phospholipids, a lipoprotein cell membrane and tight junction complexes prevents hydrogen ion back diffusion. Increased mucosal blood flow provides nutrients and oxygen to mucosal cells and removes hydrogen ions which have penetrated the gastric barrier. The rapid turnover of epithelial cells is important for rapid healing, if damage of gastric mucosa occurs. In addition, sulphydryls produced locally may act as scavengers of oxygen and other tissue damaging radicals. The cytoprotective effects of PGE_2 can be blocked by non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandins synthesis and thus have ulcerogenic effect.

DRUGS STIMULATING GASTRIC SECRETION

Several agents promote the functional activity of stomach by increasing secretion and motility. However, drugs which specifically stimulate gastric secretion have limited therapeutic use.

Classification

I. Gastrin analogues
   e.g. pentagastrin.
II. Histamine and analogues
   e.g. histamine and betazole.
III. Stomachics
   e.g. neostigmine, bethanechol, alkaline stomachics and bitters.

I. Gastrin Analogues

Pentagastrin

Pentagastrin is a synthetic pentapeptide, which has all the physiological actions of endogenous gastrin. It consists of the working end of gastrin molecule (Trp-Met-Asp-Phe) to which a substituted β-alanine has been added (Figure 42.2).

The most prominent action of pentagastrin is stimulation of gastric acid, pepsin and intrinsic factor secretion. It also stimulates pancreatic secretion, inhibits absorption of water and electrolytes, and increases blood flow in the gastric mucosa. Pentagastrin contracts smooth muscles of lower oesophageal sphincter, gall bladder, intestine and colon. Although pentagastrin contracts the smooth muscles of the lower oesophageal sphincter and stomach, it delays gastric emptying.

Pentagastrin is used as a diagnostic agent for evaluation of gastric acid secretory function i.e. to determine whether or not parietal cell function is normal (see also Chapter 27). After its SC administration, gastric secretion begins within 10 minutes, peaks within 30 minutes and lasts for about an hour.

Dose

For diagnostic purposes

Dogs : 6 µg/kg, SC.

II. Histamine and Analogues

Histamine acid phosphate and betazole (a histamine isomer) are used for clinical testing of gastric secretions. Betazole has less marked effect on blood pressure and smooth muscles than histamine and is more commonly used to stimulate gastric secretion in cases of suspected hypochlorhydria or achlorhydria. If histamine is used, an H_1-receptor antagonist should be administered 30 minutes before histamine challenge to prevent serious side effects. The pharmacology of histamine and betazole has been given in Chapter 27.

Dose

Histamine acid phosphate

Dogs : 0.04 mg/kg, SC.

Betazole

Dogs : 1.7 mg/kg, SC.

III. Stomachics

Stomachics are agents which promote the functional activity of stomach by increasing gastric secretion and motility. Several agents act as stomachics, but their therapeutic utility is limited. Cholinergic agonists such as neostigmine and bethanechol produce a stomachic effect by stimulating gastric muscarinic receptors. Alkaline stomachics including bicarbonate and carbonate salts induce gastric secretion, which in turn leads to distension of stomach and hyperaemia of mucosa. Bitters may also act as stomachics and are occasionally included in tonic formulations.

DRUGS INHIBITING GASTRIC SECRETION

Several groups of drugs are used to prevent or modulate gastric acid secretion in conditions like hyperacidity, peptic ulcers, reflux oesophagitis and Zollinger-Ellison syndrome (gastrin producing tumour). Drugs which modify gastric pH and/or provide gastric mucosal protection are discussed separately as acid neutralising and cytoprotective drugs.

Classification

Inhibitors of gastric secretion can be divided into following groups :

I. H_2-Receptor antagonists
   e.g. cimetidine, ranitidine, famotidine, nizatidine, roxatidine and loxatidine.
II. Proton pump inhibitors
   e.g. omeprazole, esomeprazole, lansoprazole, dexlansoprazole and pantoprazole.
III. Muscarinic receptor antagonists
   e.g. pirenzepine, propantheline and oxyphenonium.
IV. Prostaglandin analogues
   e.g. misoprostol, rioprostil and enprostil.

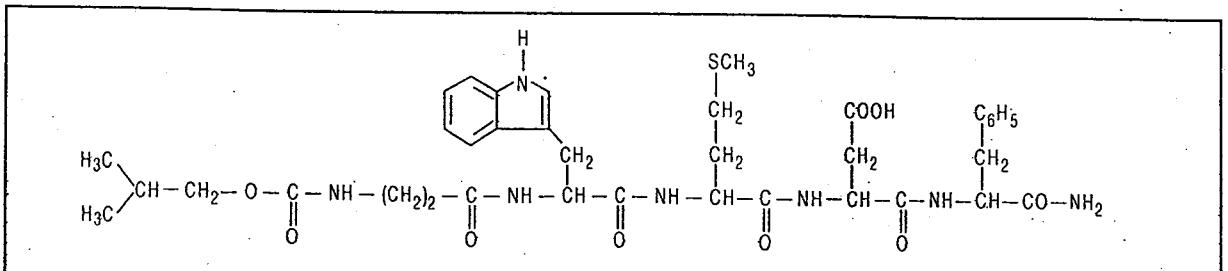


Fig. 42.2. Chemical structure of pentagastrin

I. H_2-Receptor Antagonists

The H_2 receptor antagonists are a class of drugs used to block the action of histamine on parietal cells in the stomach, decreasing the production of acid by these cells. These drugs are widely used in both human and veterinary medicines in treatment of peptic ulcer disease, gastroesophageal reflux disease and dyspepsia. Cimetidine, ranitidine, famotidine and to lesser extent nizatidine have been used to control gastric acid secretion in animals.

Structure

The H_2-receptor antagonists in clinical use are histamine congeners, which inhibit the interaction of histamine with H_2-receptors. Cimetidine structure retains the imidazole ring of histamine, which is replaced by furan in ranitidine and a thiazole in famotidine and nizatidine.

Mechanism of action

The H_2 antagonists are inhibitors of histamine at the parietal cell H_2 receptor. Although H_2 receptors are present in numerous tissues (e.g. vasculature and bronchial smooth muscle), but in stomach they are more specifically involved in gastric acid secretion. The interaction of drugs with H_2 receptors has been found to be competitive in case of cimetidine, ranitidine, nizatidine and roxatidine, competitive-non-competitive in case of famotidine, and non-competitive with loxatidine. Each drug varies in potency, duration of action, disposition and drug interactions.

Cimetidine

Cimetidine was the first H_2 receptor blocker to be introduced clinically and was described as the prototype. Cimetidine HCl occurs as white, crystalline powder and is soluble in water and alcohol. It should be stored protected from light and kept at room temperature. Cimetidine and other H_2 receptor antagonists are more hydrophilic than the H_1-receptor antagonists, so they reach the central nervous system only to a limited extent.

Pharmacological effects

Cimetidine, like other H_2-receptor antagonists, inhibits the interaction of histamine with H_2-receptors present in numerous tissues, but its most important action is observed on gastric parietal cells. At the H_2-receptors of parietal cells, cimetidine competitively inhibits histamine, thereby reducing gastric acid output both during basal conditions and when stimulated by food, pentagastrin, histamine, insulin, fundic distension and to a lesser extent by muscarinic agonists in a dose-dependent manner. It reduces both the secretion as well as the amount of pepsin induced by a variety of secretogogues. Gastric emptying time, pancreatic or biliary secretion, and lower oesophageal pressure are not altered by cimetidine. Secretion of intrinsic factor is also reduced; however, since this protein normally is secreted in great excess, absorption of vitamin B_{12} is not interfered even after long-term use of cimetidine. Cimetidine and other H_2-receptor antagonists protect experimental animals from gastric ulceration induced by stress, pyloric ligation, H_2 agonists or cholinomimetics. However, they have doubtful value for protecting dogs from GI lesion caused by NSAIDs. The H_2-antagonists can also diminish the reflux of gastric acid, but in cases of severe oesophagitis they are notably inferior to omeprazole.

Cimetidine has an apparent immunomodulating effect as it has demonstrated to reverse suppressor T-cell mediated immune suppression. It also possesses weak anti-androgenic activity, elevates prolactin levels and decreases metabolism of estradiol.

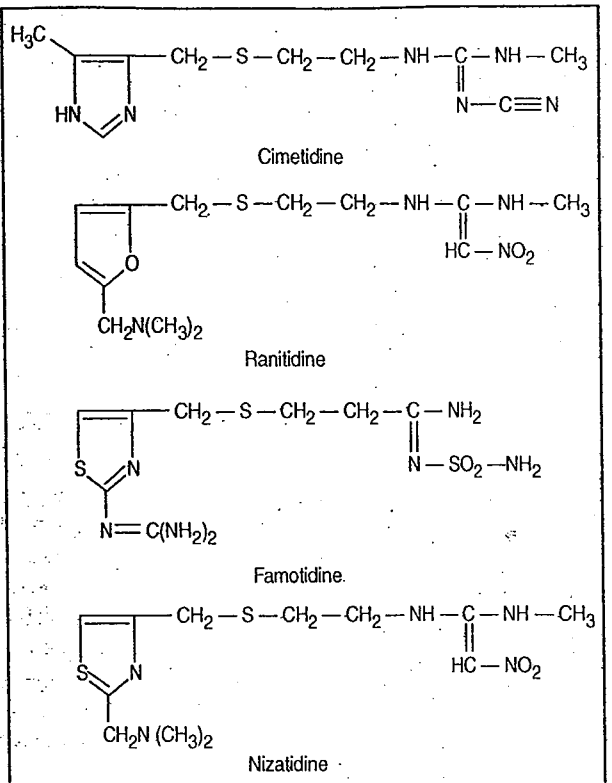


Fig. 42.3. Chemical structures of important H_2-receptor antagonists.

Pharmacokinetics

Pharmacokinetics data in domestic animals are limited for cimetidine. This drug is rapidly absorbed from the gastrointestinal tract, although food delays the process. In dogs, the oral bioavailability is reported to be approximately 95%, serum half-life is 1.3 hours and volume of distribution is 1.2 L/kg. It crosses placenta and reaches milk, but penetration into the brain is poor due to its hydrophilic nature. The drug undergoes hepatic metabolism and is excreted in urine in both unchanged and conjugated forms. In humans, its bioavailability is ~50% due to first-pass hepatic metabolism.

Side effects/Adverse effects

Side/Adverse effects of cimetidine appear to be very rare in animals at the dosage generally used. Thrombocytopenia has been reported in some animals. In laboratory animals, very high doses have been associated with tachycardia and respiratory failure. Potential adverse effects which have been documented in human beings include headache, dizziness, bowel upset, rashes, restlessness and confusion. Large IV doses can produce arrhythmias, bradycardia and cardiac arrest. As cimetidine (not other H_2-antagonists) possesses anti-androgenic action, it decreases metabolism of estradiol and increases plasma prolactin concentration. Its prolonged use may produce gynaecomastia, loss of libido, impotence and temporary decrease in sperm count. A clinically important disadvantage of cimetidine described in human patients is relapse of gastroduodenal ulceration due to rebound hypersecretion of gastric acid after the therapy is discontinued.

Drug interactions

a. Cimetidine is a known inhibitor of many isozymes of the cytochrome P450 enzyme system (specifically CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4). This

inhibition forms the basis of the numerous drug interactions which occur between cimetidine and other drugs. It decreases the hepatic metabolism of numerous drugs including theophylline, phenobarbital, warfarin, digitoxin, quinidine and phenytoin. Impaired metabolism of other drugs can lead to their accumulation to toxic levels in body.

b. Like all antisecretory drugs, cimetidine impairs oral absorption of a number of drugs (generally weak acids) due to alteration of the GI pH. Cimetidine also impairs the absorption of drugs by binding to them.

c. Cimetidine reduces hepatic blood flow by about 20% and has been shown to reduce the clearance of flow-limited drugs such as propranolol and lidocaine.

d. Cimetidine can inhibit tubular secretion of procainamide, with increase in plasma concentrations of the drug and its cardioactive metabolite N-acetylprocainamide.

e. Cimetidine may exacerbate leucopenia when used with other drugs which cause this problem.

f. Antacids and metoclopramide reduce absorption of cimetidine and other H_2-receptor antagonists. If used concurrently, a gap of 2 hours should be allowed between the dosing of two drugs.

Clinical uses

In veterinary medicine, cimetidine has been used for the treatment and prophylaxis of gastric, abomasal and duodenal ulcers and in uraemic gastritis, stress related or drug induced erosive gastritis, duodenal gastric reflux and oesophageal reflux. Cimetidine is employed to treat hypersecretory conditions associated with gastrinomas and systemic mastocytosis. It is also used as adjunct in exocrine pancreatic insufficiency, where if given about 30 minutes prior to feeding it may decrease enzymatic and acid hydrolysis of replacement pancreatic enzymes added to the food. Cimetidine has also been used in the treatment of equine melanomas. Cimetidine and ranitidine also appear to be effective in controlling upper gastrointestinal bleeding, when haemorrhage is not due to erosion of major blood vessels.

Dose

For gastric ulceraton, oesophagitis or gastric hyperacidity.

Dogs & cats : 5 – 10 mg/kg, PO, SC, IM, or slow IV (over a period of 20-30 minutes), 3 to 4 times daily.

For abomasal ulceration

Cattle : 8 – 16 mg/kg, 3 times daily.

Gastric ulceration

Foals : 20 mg/kg, PO, 2 times daily.

: 5 mg/kg, IV, 4 to 6 times daily.

Swine : 330 mg (total), 2 times daily.

For uraemic gastritis

Dogs & cats : 2.5 – 5 mg/kg, PO or slow IV, 2 to 3 times daily.

For equine melanoma

Horses : 2.5 mg/kg, PO, 3 times daily for 3 months.

Ranitidine

Ranitidine HCl is a competitive H_2-receptor antagonist widely used to inhibit gastric acid secretion. It was introduced subsequent to cimetidine as a non-imidazole (has a furan ring) H_2-blocker and has several desirable features compared to cimetidine.

Pharmacological effects

Ranitidine is a H_2-receptor antagonist that possesses pharmacological properties similar to those of cimetidine. However, it is 5 to 12 times more potent as an inhibitor of gastric acid secretion than cimetidine and does not significantly inhibit hepatic metabolism of other drugs. Ranitidine, unlike cimetidine, does not appear to have any appreciable effect on serum prolactin levels and it lacks anti-androgenic effect. Ranitidine stimulates GI motility and may be useful as prokinetic agent in the treatment of gastric emptying disorders as well as gastric ulcers. Unlike cimetidine, ranitidine is not known to inhibit microsomal P450 enzymes.

Pharmacokinetics

Pharmacokinetics data on ranitidine are limited for animals. In dogs, its oral bioavailability is approximately 80%, serum half-life is 2.2 hours and volume of distribution is 2.6 L/kg. Food does not appreciably alter the extent of absorption or the peak serum levels attained. The serum half-life of ranitidine in man ranges from 2 to 3 hours and duration of action is from 8 to 12 hours. Ranitidine is distributed into human milk at levels of 25-100% of those found in the plasma.

Side effects/Adverse effects

Side effects of ranitidine appear to be very rare in animals at the dosages generally used. Although some side effects (e.g. confusion, headache, diarrhoea/constipation) have been reported in human beings, limited clinical experience in animals have not indicated any serious toxic manifestation from ranitidine. Some rare serious adverse effects like CNS manifestations and haematological changes have been reported in elderly and seriously ill human patients.

Contraindications and precautions

Ranitidine should not be used in animals with known hypersensitivity or allergy to the drug. Ranitidine should be avoided in animals with liver or kidney disease.

Drug interactions

Unlike cimetidine, ranitidine appears to have only minimal effects on the hepatic metabolism of drugs and is unlikely to cause clinically relevant drug interaction via this mechanism. It has limited to no effect on hepatic blood flow. The relative bioavailability of ranitidine may be increased with high peak serum level when propantheline is administered concomitantly with ranitidine. Antacids may decrease absorption of ranitidine.

Clinical uses

The clinical uses of ranitidine are similar to those of cimetidine i.e. in gastric and duodenal ulceration, reflux oesophagitis and hypersecretory conditions. Dogs and cats with mast cell tumours may be treated with ranitidine or a related drug because these tumours can produce large amounts of histamine. Presently, ranitidine appears to more commonly used than cimetidine.

Dose

Dogs & cats : 0.5 – 2 mg/kg, PO, IV, IM, or SC, 2 to 3 times daily.

Horses : 4 – 6 mg/kg, PO, 2 times daily.

: 1 mg/kg, IV, 3 times daily.

Famotidine

Famotidine is a thiazole ring containing H_2 antagonist, which occurs as a white to yellow, crystalline powder. Like cimetidine, it reduces gastric acid output both during basal conditions and when stimulated by food, pentagastrin, histamine or insulin. Famotidine is about 9 times more potent than ranitidine and about 32 times more potent than cimetidine as an inhibitor of gastric acid secretion. Further, it appears to act competitively/non-competitively at the H_2 receptors. It has no anti-androgenic action and has low affinity for cytochrome P-450 enzyme system. Unlike ranitidine, it has low prokinetic activity.

Famotidine is only 37% bioavailable after oral administration due to poor oral absorption, however, it undergoes minimal first-pass metabolism. It exhibits longer duration of action despite an elimination t½ of 2-3 hours. Distribution characteristic are not well defined. Because there is limited experience with this drug, the adverse effects profile of famotidine has not been determined for domestic animals. It appears to be devoid of many of the side effects of cimetidine. Very high doses may cause hypotension, tachycardia and collapse. Intravenous solution contains benzyl alcohol; therefore IV injection to small animals, especially cats, should be done slowly.

Similar to cimetidine, famotidine is clinically used for gastric hypersecretory conditions. Because of the high potency and longer duration, it has been considered more suitable for Zollinger-Ellison syndrome and for prevention of aspiration pneumonia in humans.

Dose

For gastric hypersecretory conditions

Dogs & cats : 0.5 – 1 mg/kg, PO, SC, IM, or IV, 1 to 2 times daily.

Horses : 1 – 2 mg/kg, PO, 3-4 times daily.

As an adjunct in ulcer treatment

Horses : 0.35 mg/kg, IV, 2 times daily.

: 2.8 mg/kg, PO, 2 times daily.

Nizatidine

Nizatidine is another thiazole ring containing H_2-receptor antagonist, which acts in a manner similar to that of cimetidine and ranitidine. It competitively blocks gastric secretions and is more potent than cimetidine. It also stimulates gastric contractions and accelerates gastric emptying (prokinetic effect).

Nizatidine is rapidly and competitively absorbed after oral administration and largely eliminated unchanged in urine. Nizatidine is almost exclusively eliminated by renal excretion, which suggests that it may be preferentially used in patients with hepatic diseases. Nizatidine is considered to be devoid of many of the side effects of cimetidine. As nizatidine does not inhibit hepatic microsomal enzymes, chances of drug interactions involving the metabolism are rare. Among various H_2-receptor blockers studied, nizatidine and famotidine appears to cause minimal rebound gastric acid hypersecretion.

Dose

Dogs : 1-5 mg/kg, PO, once daily.

Other drugs

Roxatidine, loxatidine and zolentidine are other H_2-receptor antagonists, mainly used in human medicine. The pharmacodynamics, pharmacokinetics and side effect profile of roxatidine are similar to those of ranitidine, but it is twice as potent and longer acting. Loxatidine is a powerful non-competitive H_2-receptor antagonist, which can produce almost complete inhibition of gastric secretion. Zolentidine is a highly lipophilic H_2-antagonist that is under study for its potential ability to antagonise histamine in the CNS; it has little or no effect on gastric acid secretion. The clinical efficacy of these drugs has not been studied in veterinary species.

II. Proton Pump Inhibitors

Proton pump inhibitors (PPIs) are a group of drugs which irreversibly block the H^+-K^+-ATPase (Proton pump) in the gastric parietal cells and produce a pronounced and long-lasting reduction of gastric acid production. These drugs are largely given in an inactive form, which in acidic environment of parietal cells become activated to cause blockade of gastric proton pump. Proton pump inhibitors are the most potent inhibitors of acid secretion available today.

Omeprazole

Omeprazole is the prototype member of a new class of substituted benzimidazoles which inhibit the proton pump (H^+-K^+-ATPase enzyme system) of parietal cells, thereby suppressing secretion of hydrogen ions into the gastric lumen. Omeprazole inhibits acid secretion during both basal and stimulated conditions without much effect on pepsin, intrinsic factor, juice volume and gastric motility.

Mechanism of action

Omeprazole is a racemate, which can exist in equal amounts of both the S and R enantiomers. Omeprazole and other proton pump inhibitors contain a sulfinyl group in a bridge between substituted benzimidazole and pyridine rings. As a weak base of pK_a 4.0, omeprazole is inactive, stable and lipid soluble at neutral pH. After oral administration, omeprazole in inactive form passes into the small intestine, where in the more alkaline environment the drug dissolution and absorption occurs. After absorption, omeprazole selectively partitions from systemic circulation into parietal cells and diffuses into the secretory canaliculi which have acidic environment. In the acid environment, the inactive drug becomes protonated and gets trapped (charged form generated at acidic pH is unable to diffuse back). The protonated agent then rearranges itself and gets activated to first form a sulphenic acid and then a sulphenamide. The sulphenamide formed interacts covalently with the sulphydryl group (-SH group) of a cysteine group in the H^+-K^+-ATPase enzyme and inactivates it irreversibly (Figure 42.4). This causes permanent inhibition of the enzyme; secretions of hydrogen chloride (HCl) resumes only after new molecules have been formed in the luminal membrane.

Because the drug accumulates in parietal cells, there may be a lag time of 3 to 5 days before maximum effect is realised and efficacy is maintained for some time after the drug is discontinued. The specificity of effects of proton pump inhibitors derive from the selective distribution of H^+-K^+-ATPase, requirement for acidic conditions to catalyse generation of reactive inhibitor, and trapping activated form of the drug within acidic canaliculi.

Pharmacological effects

At standard doses, omeprazole inhibits basal and stimulated gastric acid secretion more than 90% without anticholinergic or

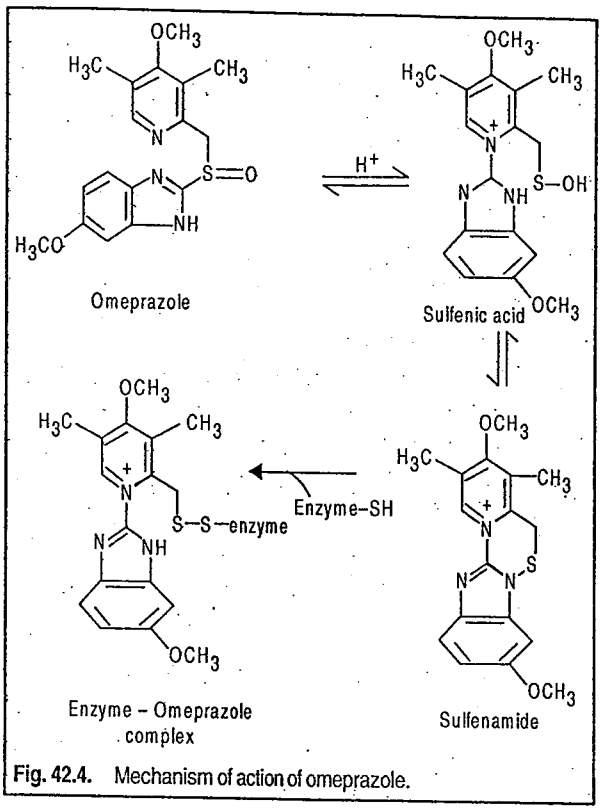


Fig. 42.4. Mechanism of action of omeprazole.

H_2-receptor blocking action. It produces only small and inconsistent changes in the gastric juice and in the secretion of pepsin and intrinsic factor, and does not affect gastric motility. In dogs, it is approximately 10 times more potent than cimetidine and can achieve virtual gastric antacidity something that is difficult with H_2-receptor antagonists.

Pharmacokinetics

Omeprazole is available for oral administration in enteric-coated granule form as the drug is rapidly degraded by gastric acid. If taken appropriately, enteric-coated capsule releases omeprazole after the granules leave the stomach, and the drug is rapidly absorbed from the intestine. In humans, omeprazole is highly (96%) bound to serum albumin and α_1-acid glycoprotein.

Omeprazole is extensively metabolised in the liver to inactive metabolites, which are excreted mainly in urine and also via bile into faeces. Though its plasma half-life is short (1 hour), yet it can be detected in the gastric mucosa after its disappearance from plasma because of its tight binding to the target enzyme. As such, inhibition of HCl secretion occurs within 1 hour, reaches maximum at 2 hour and lasts for about 3 days. With daily administration, antisecretory effect increases till 4th day after which it plateaus. Gastric secretion resumes gradually over 3-5 days of stopping the drug probably due to synthesis and incorporation of new molecules of H^+-K^+-ATPase into the membrane.

Side effects/Adverse effects

Omeprazole has been studied in dogs and cats and found to be safe at normal dosages. Gastrointestinal disturbances like anorexia, colic, nausea, vomiting, flatulence and diarrhoea could occur. Prolonged administration of very high doses of omeprazole to experimental animals has been reported to cause hyperplasia of oxyntic mucosal cell due to increased secretion of gastrin, which has tropic effect on these cells. Its prolonged use, therefore, is not recommended.

Drug interactions

Omeprazole, unlike cimetidine, is less involved in drug interactions. Omeprazole also inhibits some cytochrome P-450 iso-enzymes, so it may decrease the hepatic clearance of drugs (e.g. warfarin and phenytoin) those are metabolised by microsomal enzyme and may enhance their toxicity. Absorption of drugs (e.g. ketoconazole and itraconazole) which require low pH may be reduced by omeprazole as it elevates gastric pH.

Clinical uses

Omeprazole is the drug of choice for the treatment of Zollinger-Ellison syndrome. It has been used to control disorders associated with gastric acid secretion that has not responded to H_2-receptor antagonists. Omeprazole is reported to be superior to cimetidine for treatment of gastrointestinal ulceration. It is also useful in reflux oesophagitis in dogs and cats. Omeprazole is often combined with the antibiotics for eradication of *Helicobacter pylori*, the causative factor in majority of the peptic and duodenal ulcers. In ruminants, the oral absorption of omeprazole may not be high enough for effective therapy.

Dose

For ulcer management

Dogs & cats : 0.7 mg/kg, PO, once daily.
Horses : 0.5 – 2 mg/kg, once daily.

Esomeprazole

Esomeprazole is the S-enantiomer of omeprazole claimed to have higher bioavailability and improved efficacy over the racemic mixture of omeprazole. Its mechanism of action and pharmacological properties are similar to those of omeprazole. It is reported to produce better control of intragastric pH than omeprazole in gastroesophageal reflux disease (GORD/GERD) patients because of better pharmacokinetic profile. The drug is cleared from body, largely by the urinary excretion of pharmacologically-inactive metabolites such as 5-hydroxymethylesomeprazole and 5-carboxyesomeprazole. Esomeprazole may inhibit CYP2C19 and CYP2C9 isozymes and may therefore interact with drugs which depend on them for metabolism, such as diazepam and warfarin. Side/Adverse effects and clinical uses of esomeprazole are similar to those of the racemic drug omeprazole.

Lansoprazole

Lansoprazole is a proton pump inhibitor with mechanism of action and pharmacological properties similar to those of omeprazole. Similar to omeprazole, lansoprazole is a racemate (1:1-mixture of the enantiomers dexlansoprazole and levolansoprazole). The potency of lansoprazole is somewhat more than omeprazole, but the inhibition of acid secretion may not be persistent as its action on proton pump is partly reversible. Lansoprazole has higher oral bioavailability and slightly longer half-life than omeprazole. Side effects of lansoprazole are similar but drug interactions appear to be less significant and it does not interfere with the metabolism of other drugs. There is limited experience with lansoprazole in veterinary medicine.

Dexlansoprazole

Dexlansoprazole is the dextro-enantiomer of lansoprazole. Its mechanism of action, pharmacological effect and side-adverse effects profile are similar to those of lansoprazole. Dexlansoprazole has been approved for use in the treatment and maintenance of

patients with erosive oesophagitis and non-erosive gastro-oesophageal reflux disease (GORD /GERD).

Other drugs

Pantoprazole, S-pantoprazole and rabeprazole are some newer proton-pump inhibitors, similar in action, properties and clinical efficacy to omeprazole. Pantoprazole, a racemate, is more acid stable than omeprazole and has higher oral bioavailability. S-pantoprazole is the active single enantiomer of pantoprazole, but is twice as potent as the racemate. Rabeprazole is claimed to cause fastest acid suppression action due to its higher pKa and rapid conversion to active form.

III. Muscarinic Receptors Antagonists

1. Non-Selective Muscarinic Receptor Antagonists

Non-selective muscarinic receptor antagonists such as atropine, glycopyrronium, aminopentamide, propantheline and oxyphenonium can reduce gastric motility and basal secretion without raising the pH of stomach. Stimulated gastric secretion, however, is less completely inhibited. These drugs also delay gastric emptying and allow antacids given concurrently to act for longer period. Non-selective muscarinic receptor antagonists are of lesser importance in animals than man because gastric secretion is not as continuous. These drugs also have many intolerable side effects.

2. Selective M_1-Receptor Antagonists

Selective antagonists of M_1-receptors are as effective as atropine or other non-selective muscarinic antagonists in inhibiting gastric secretion. Further, they are less likely to produce the adverse effects those are characteristic of cholinergic blockade.

Pirenzepine and Telenzepine

Pirenzepine and telenzepine are relatively specific muscarinic M_1-receptor antagonists, which have renewed interest in this class of antisecretory drugs. They reduce basal and stimulated gastric secretion at doses which have minimal effects on the heart, eyes and bladder. Although both drugs are less effective than H_2-receptor antagonists in reducing acid secretion, they are reported to be as effective as cimetidine in healing duodenal and gastric ulcers. They are believed to act on M_1 receptors found in parasympathetic ganglia and possibly also on histamine secretory cells and gastric parietal cells. Both drugs are hydrophilic and penetrate blood-brain barrier poorly.

Telenzepine is more potent than pirenzepine and is effective at lower dosage. Both drugs produce cholinergic side effects (dry mouth, constipation and blurred vision) in slightly higher dosage, which may limit their utility. These drugs are mostly used as an adjunct to the H_2-receptor antagonists in human beings for the treatment of peptic ulcers. The detailed pharmacology of muscarinic receptor antagonists have been discussed in Chapter 14.

IV. Prostaglandin Analogues

Prostaglandins E_2 and I_2 (PGE_2 and PGI_2) are produced in the gastric mucosa and appear to serve a cytoprotective action by inhibiting acid secretion, promoting mucus and bicarbonate secretion and increasing mucosal blood flow. Prostaglandins also inhibit gastric production and help to reinforce the mucus layer covering GI mucosa and stimulating cell turnover and repair when epithelium is damaged. Because administration of PGs protects the gastric mucosa of animals against various ulcerogenic insults, a number of prostaglandin analogues have been developed and tested in human beings and animals.

Misoprostol

Misoprostol is a methyl ester analogue of prostaglandin E_1. It inhibits basal, nocturnal and food-induced gastric secretion, but at doses slightly higher than those required to increase secretions of mucus and HCO_3^- and produce cytoprotective action. Its action appears to be mediated by interaction with a basolateral membrane receptor, which decreases concentration of cAMP with subsequent decrease in protein kinase activity and hydrogen ion concentration.

Misoprostol is very effective in preventing GI ulcers and erosions caused by NSAIDs. Its routine prophylactic use may not be justified except in patients taking NSAIDs those are at high risk of NSAID-induced ulcers. Misoprostol is not generally used to treat existing ulcers because it is not a H_2-antagonist and does not relieve ulcer pain, while at the same time it seems to have more side effects than other drugs.

In veterinary medicine, the indications for using misoprostol in dogs are currently uncertain. It is recommended in dogs with severe osteoarthritis which require NSAIDs to be functional but those cannot tolerate NSAIDs because of subsequent gastric problems. It is less effective in gastric ulcer and reflux oesophagitis. The pharmacology of misoprostol has been described in Chapter 29.

Other prostaglandin analogues

Some other congeners of PGE_1 (e.g. rioprostil) and PGE_2 (e.g. arboprostil, enprostil and trimoprostil) are also being used for cytoprotective actions in human beings, but they are under study in animals.

ACID NEUTRALISING AND CYTOPROTECTIVE DRUGS

I. ACID NEUTRALISING DRUGS /ANTACIDS

Acid neutralizing drugs or antacids are a group of basic substances which are used to neutralize gastric acid and increase pH of gastric contents. Since pepsin is inactivated at pH >4.0, antacids also reduce peptic activity. In addition, they may have mucosal protecting action i.e. they enhance bicarbonate secretion, increase PGs and sulphydryl synthesis, and decrease *Helicobacter pylori* colonisation. Inactivation of pepsin and binding of bile salts by some agents (e.g. aluminium hydroxide) is also important. Antacids have no effect on the gastric parietal cells which secrete HCl. Therefore, they do not decrease acid production, rather some agents which increase the antral pH to >4.0 evoke reflex gastrin release and subsequent more acid secretion called 'acid rebound condition'. Antacids are less popular in veterinary medicine because they require frequent administration to produce continuous acid neutralisation and to prevent acid rebound condition.

Mechanism of action

Antacids diminish the quantity of free hydrochloric acid in stomach by 3 importants methods viz. direct neutralisation of pre-formed acid (e.g. sodium bicarbonate and magnesium hydroxide), buffering of pre-formed acid (e.g. magnesium trisilicate and sodium

citrate), and adsorption of H^+ and adsorption and inactivation of pepsin (e.g. aluminium antacids and anion exchange resins). The potency of antacids is generally defined in terms of its acid neutralising capacity (ANC), which is defined as the numbers of mEq of 1 N HCl which are brought to pH 3.5 in 15 minutes (or 60 minutes in some tests) by a unit dose of the antacid preparation. This takes into account the equivalent weight of the compound and the rate at which it dissolves and reacts with HCl. The acid-neutralising ability of an antacid depends on its capacity to neutralise gastric HCl and on whether stomach is full or empty (because food delays gastric emptying, allowing more time for the acid to react).

Classification

Antacids products vary widely in their chemical composition, acid-neutralising capacity, and side effects. Broadly, these have been divided into 2 groups: systemic antacids and non-systemic antacids.

1. Systemic antacids/Fast acting antacids
   e.g. sodium bicarbonate and sodium citrate.
2. Non-systemic antacids/Modest to slow acting antacids
   e.g. aluminium hydroxide, magnesium hydroxide, magnesium trisilicate, magnesium carbonate, calcium carbonate and magaldrate.

1. Systemic Antacids/Fast Acting Antacids

The systemic antacids are those acid neutralizing agents which neutralize acidity in stomach and thereafter are absorbed to cause systemic effects such as alkalosis. Hence these agents are likely to disturb the acid base balance is body.

Sodium bicarbonate

Sodium bicarbonate (Sodium hydrogen carbonate, baking soda) occurs as white, crystalline powder having a slightly saline or alkaline taste. It is soluble in water and insoluble in alcohol. Sodium bicarbonate powder is stable in dry air, but it slowly decomposes upon exposure to moist air.

Mechanism of action

Sodium bicarbonate is a base, so it directly neutralises pre-formed acid in the stomach. It increases the gastric pH to more than 4.0, which is not stronger enough to cause damage to the mucosal surface.

$$NaHCO_3 + HCl \longrightarrow NaCl + H_2O + CO_2$$

Pharmacological effects

On oral administration, sodium bicarbonate produces instantaneous action and offers rapid relief from pain. It is a potent neutraliser of acid i.e. 1 g neutralises about 12 mEq of HCl. However, its duration of action is short due to its systemic absorption. Sodium bicarbonate has several limitations as antacid, so it should not be used in excess or for a long-term period.

Side effects/Adverse effects

Oral administration of sodium bicarbonate makes the gastric pH almost alkaline (> 7.0) that may interfere with the peptic digestion of food (pepsin requires pH of 4.0 for its action, and at >5.9 its activity decreases). High gastric pH increases gastrin release, which in turn increases HCl secretion due to acid rebound condition. Rapid release of CO_2 may produce distension of stomach, which stimulates more secretion of gastric acid. Excess of carbon dioxide also produces discomfort, belching and risk of ulcer production. Excessive administration of sodium bicarbonate may result in metabolic alkalosis and worsen the oedema and CHF due to increased Na^+ load in the body. Absorbed CO_2 also is converted to bicarbonate in body leading to acid-base imbalance that is usually transient and clinically insignificant in patients with normal renal function.

Contraindications and Precautions

Important contraindications and precautions of sodium bicarbonate are mentioned in Chapter 41.

Drug interactions

Oral administration of sodium bicarbonate can alter (increase or decrease) the rate and/or extent of absorption of many orally administered drugs by enhancing the gastric pH. Therefore it is recommended to avoid giving other drugs within 1-2 hours of sodium bicarbonate administration. Sodium bicarbonate decreases absorption of antimuscarinic drugs, H_2-antagonists, tetracyclines, iron products and ketoconazole, and increases dissolution and absorption of levodopa, valproic acid and acidic form of sulphonamides. Alkaline urine produced by sodium bicarbonate delays excretion of certain drugs and hastens excretion of weakly acidic drugs (see for detail Chapter 41). Sodium bicarbonate should not be taken along with milk and milk products as it increases Ca^{++} absorption leading to hypercalcaemia.

Clinical uses

Sodium bicarbonate provides quick symptomatic relief from acidity, so its use is restricted mainly to casual treatment of hyperacidity and epigastric pain. Sodium bicarbonate is also indicated to treat metabolic acidosis, to alkalinise urine and as adjunctive therapy in treating hypercalcaemic or hyperkalaemic crisis (see for detail Chapter 40). Sodium bicarbonate is not suitable for use in peptic ulcers.

Dose

As an antacid

Cattle	:	15-30 g (total), PO, 2 to 3 times daily.
Horses	:	8 – 15 g (total), PO, 2 to 3 times daily.
Dogs	:	0.3 – 1.3 g (total), PO, 2 to 3 times daily.

Sodium citrate

Sodium citrate is an odourless white crystalline powder or granular crystal having a saline taste. It is soluble in water and used as an alkalizer of blood and urine (see Chapter 41), antacid and anticoagulant (see Chapter 35). As antacid, its properties are similar to those of sodium bicarbonate. However, it does not release CO_2. One gram of sodium citrate neutralises about 10 mEq of HCl.

2. Non-Systemic Antacids

The non-systemic antacids are those acid neutralizing agents which decrease acidity in stomach, but are not/minimally absorbed after administration. Unlike systemic antacids, the non-systemic agents carry minimal risk of acid base imbalance. The major non-systemic antacids used in veterinary medicine are salts of aluminium, magnesium and calcium used either alone or in combination with each other or with various protectants, absorbents and astringents.

Mechanism of action and Pharmacological effects

Non-systemic antacids are insoluble and poorly basic compounds, which react with acid in stomach to neutralise the

acidity. In the reaction, they form the corresponding chloride salt (see individual drug), which largely reacts with the intestinal bicarbonate and remains unabsorbed. Small amounts which are absorbed have the same alkalinising effect as sodium bicarbonate. Among various antacids, magnesium salts tend to be laxative, and aluminium and calcium salts have constipating effects. Therefore, they are mostly used in combinations, so as to avoid adverse effect on gastrointestinal motility. The duration of action of various antacids depends on gastric emptying time, volume of acid secreted and respective neutralising capabilities.

Drug interactions

Similar to sodium bicarbonate, non-systemic antacids show several drug interactions. By altering gastric and urinary pH, they can alter rates of dissolution and absorption, bioavailability and renal elimination of a number of drugs. They can decrease the absorption and pharmacological effects of several drugs like cimetidine, corticosteroids, digoxin, iron salts and phenothiazines, and increase absorption and pharmacological effects of dicoumarol, quinidine and sympathomimetics. Orally administered tetracycline products can be chelated and prevented from being absorbed by antacids. Most drug interactions can be avoided by taking antacids 2 hours before or after ingestion of other drugs.

Clinical uses

In veterinary medicine, non-systemic antacids are useful in the treatment of acute gastritis, reflux oesophagitis, chronic renal failure (uraemia), gastric and duodenal ulceration, hypophosphataemia and in cases recovering from acute gastric dilation. In ruminants, their main use is to counter the acidity of rumen overload syndrome (acute rumen engorgement, rumen acidosis, and grain over-load and engorgement toxaemia). While antacids alone may be sufficient in mild ruminal acidosis, more severe cases may require administration of intravenous sodium bicarbonate and isotonic fluid therapy. Presently, the antacids have largely been relegated to adjunctive roles in the therapy for various indications in foals and small animals because of difficulty in administration, frequent dosing and availability of H_2 antagonists. However, these agents are still considered important in reducing hyperphosphataemia in patients with renal failure.

The antacids are best given between meals and at night time to dogs and cats. The reduced frequency of administration of antacids (compared with man) in small animals is due to intermittent rather than continuous secretion of acid in these species.

A. Aluminium Salts

Aluminium hydroxide

Aluminium hydroxide (Aluminium hydrate, hydrated alumina) is the prototype aluminium compound and is the most commonly used agent of non-systemic antacids group. It is obtained as a white bulky amorphous powder that is insoluble in water but soluble in alkaline aqueous solution or hydrochloric acid. It produces a gel on prolonged contact with water. In the gel form, it behaves as a non-absorbable buffer antacid with slow onset of action (so longer acting) and a low neutralising capacity (1 g neutralises about 1-2.5 mEq of HCl depending on its preparation). Its neutralising action is markedly slowed by food in the stomach.

$$Al(OH)_3 + 3\ HCl \longrightarrow AlCl_3 + 3\ H_2O$$

Aluminium hydroxide has some advantages like it maintains the gastric pH at range of 3.5-4.0. It neutralises HCl, adsorbs and inactivates pepsin, and helps to increase gastric mucus production. It is also a demulcent and has been claimed to coat and protect the ulcer crater. It also possesses adsorbent, astringent and gastrointestinal protective action. Unlike sodium bicarbonate, the acid rebound is minimal and it does not produce systemic effects.

Aluminium hydroxide relaxes smooth muscles, so it delays gastric emptying. It frequently causes constipation due to its smooth muscle relaxant and mucosal astringent action; so it is often mixed with magnesium salts to prevent this side effect. It binds phosphate in the intestine and prevents its absorption; so prolonged use may cause hypophosphataemia and osteomalacia in patients. Due to this property, aluminium hydroxide is occasionally used to control phosphate levels in the blood of individuals suffering from hyperphosphataemia in kidney failure and also in the treatment of phosphate stones.

Dose

For adjunctive therapy for gastric/abomasal ulcer and as antacid

Dogs	:	200 – 500 mg (total), PO, 4 to 6 times daily.
Cats	:	100 – 200 mg (total), PO, 4 to 6 times daily.
Dogs & cats	:	0.25 – 0.5 ml/kg of aluminium hydroxide gel (~40 mg Al_2O_3/ml), PO, 4 to 6 times daily.
Cattle	:	15 – 30 g (total) of aluminium hydroxide gel, PO, 2 to 3 times daily.
Sheep	:	1 – 2 g (total) of aluminium hydroxide powder, PO, 2 to 3 times daily.

Other salts

Other salts of aluminium like phosphate, aminoacetate, glycinate, silicate and carbonate are also used clinically and possess antacid properties similar to those of aluminium hydroxide.

B. Magnesium Salts

Magnesium hydroxide

Magnesium hydroxide is an inorganic compound with the chemical formula $Mg(OH)_2$. Magnesium hydroxide is very slightly soluble in water, so its suspension in water is often called milk of magnesia because of its milk-like appearance. The aqueous suspension (milk of magnesia) has low concentration of OH^- ions and thus low alkalinity. However, it reacts with HCl promptly and is an efficacious antacid. One gram of $Mg(OH)_2$ neutralises about 30 mEq of HCl, with formation of magnesium chloride as a product.

$$Mg(OH)_2 + 2\ HCl \longrightarrow MgCl_2 + 2\ H_2O$$

In contrast to magnesium hydroxide, the magnesium chloride formed in GI tract is soluble. However, the Mg^{++} ions are poorly absorbed from the gut and do not produce systemic effects. The soluble but unabsorbed magnesium chloride remains in the intestine and retains water. This produces an osmotic purgative action. Due to this adverse effect, magnesium antacids are often mixed with calcium or aluminium salts to avoid diarrhoea.

Magnesium hydroxide and other salts of magnesium have some limitations like they can raise gastric pH higher than aluminium-containing antacids. After administration, up to 20% of the magnesium is absorbed in normal circumstances which can result in hypermagnesemia, particularly in presence of renal dysfunction and repeated administration. Therefore, magnesium

antacids are contraindicated in renal diseases and in dialysis patients. Magnesium hydroxide and some of its salts do not release CO_2, so the rebound acidity is brief and mild. Due to their slow/moderate action, these drugs have relatively long and persistent duration of action.

Dose

For gastric/abomasal ulcers

Dogs : 5 – 30 ml (total) of milk of magnesia, PO, 1 to 2 times daily.

Cats : 5 – 15 ml (total) of milk of magnesia, PO, 1 to 2 times daily.

Cattle : up to 1 g/kg, PO, 2 to 3 times daily. Drug is usually mixed in 10-15 litres of warm water and given by tube.

Sheep & goats : 125 – 250 mg/kg, PO, 2 to 3 times daily.

Magnesium trisilicate

Magnesium trisilicate is a hydrated magnesium silicate. It has low solubility and reactivity i.e. 1 g can react with 10 mEq HCl, but in clinical practice much lesser acid is neutralised before it is passed into the duodenum. The reaction is slow and prolonged and the pH of stomach seldom rises above 3.0.

$$2MgO.3SiO_3 \times H_2O + 4HCl \longrightarrow 2MgCl_2 + 3SiO_2 + (x + 2)H_2O.$$

$$MgCl_2 + 2\ NaHCO_3 \longrightarrow MgCO_3 + 2\ NaCl + H_2O + CO_2$$

Hydrated silicic acid produced in the above reaction is gelatinous in consistency and has good adsorbent property. It is claimed to adsorb and inactivate pepsin. It reacts with gastric contents to give gelatinous silicon dioxide, which protects ulcerated mucosal surfaces and favour healing. However, contribution of these actions to clinical benefit is uncertain. Magnesium chloride formed reacts with bicarbonate in the intestine to form $MgCO_3$, which is eliminated in the faeces. About 5% of administered magnesium is absorbed systemically, which may cause problem if renal function is inadequate. Like other Mg salts, magnesium trisilicate possess purgative/laxative action due to formation of osmotically active $MgCl_2$ in the stomach and through Mg^{++} ion induced cholecystokinin release.

Dose

For abomasal ulcers (adjunctive therapy)

Cattle : Up to 16 g (total), PO.

Magnesium carbonate

Magnesium carbonate ($MgCO_3$) is a crystalline water-soluble salt that reacts with HCl at a slower rate. It generates CO_2 but its release is very gradual. It is occasionally used as a non-systemic antacid.

Dose

For abomasal ulcers (adjunctive therapy)

Cattle : Up to 16 g (total), PO.

C. Calcium Salts

Calcium carbonate

Calcium carbonate (Chalk) is a white, odourless and tasteless powder. On oral administration, it acts as an antacid, adsorbent and antidiarrhoeal drug; externally chalk is antiseptic and desiccant. As an antacid, it acts rapidly (not as rapid as sodium bicarbonate) with a prolonged duration. It has chalky taste and 1 g of it neutralises about 20 mEq HCl. The calcium chloride formed is converted in the intestine to insoluble salts (carbonate and phosphate) and calcium soaps.

$$CaCO_3 + 2\ HCl \longrightarrow CaCl_2 + CO_2 + H_2O$$

After oral administration, calcium absorption may occur up to 30%, which can produce mild degree of hypercalcaemia. Hypercalcaemia, hypercalciuria, alkalosis and formation of calcium stones in the kidney can occur on long-term use, which can be dangerous in renal insufficiency. Calcium carbonate liberates CO_2 in the stomach and can cause distension and discomfort, but the rate at which the gas is released is much slower than that produced by $NaHCO_3$.

Presently calcium salts are less used as antacids because Ca^{++} ions have shown to increase HCl production directly by parietal cells as well as by releasing gastrin due to activation of a Ca^{++} dependent process. Calcium carbonate is constipating in many individuals, but in some cases it can cause loose motion. Calcium carbonate should not be given along with milk for peptic ulcers because it may produce a milk-alkali syndrome characterised by abdominal discomfort, anorexia, weakness and abnormal Ca^{++} deposits. This syndrome results from excess Ca^{++} and absorbable alkali which cause hypercalcaemia, reduced secretion of parathyroid hormone, retention of phosphate, precipitation of Ca^{++} salts in kidneys and renal insufficiency.

Dose

Dogs : 5 – 10 ml (total), PO, 4 to 6 times daily.

Cattle : 60 – 360 g (total), PO.

Sheep : 10 – 20 g (total), PO.

Magaldrate

Magaldrate (Magnesium aluminate monohydrate) is a hydrated complex of magnesium and aluminium. It initially reacts rapidly with acid and releases aluminium hydroxide, which then reacts slowly. The freshly released aluminium hydroxide is in the unpolymerised more reactive form. Its acid neutralizing capacity (ANC) is estimated to be 28 mEq/g. It is a good antacid with prompt and sustained neutralising action and is considered better than a physical mixture of magnesium and aluminium hydroxide. Magaldrate is commonly used for the treatment of duodenal and gastric ulcers, oesophagitis from gastroesophageal reflux.

Antacid Combinations

A combination of two or more antacids is frequently used in therapeutics because the combination provides some advantages over use of any single agent.

a. Combination of magnesium/calcium salts (laxative effect) and aluminium salts (constipating effect) neutralise each other's action and gut movement is least affected.

b. Combination of fast acting (e.g. magnesium hydroxide) and slow acting (e.g. aluminium hydroxide) compounds yield rapid as well as sustained effect.

c. Combination of antacids decreases dose of individual components, thus reducing their systemic adverse effects.

Dose

Aluminium/Magnesium hydroxide suspension

For gastroduodenal ulcers

Foals : 15 ml (total), PO, 4 times daily.

II. CYTOPROTECTIVE DRUGS

Cytoprotection is a process by which chemical compounds provide protection to cells against harmful agents. Gastric cytoprotective drugs protect GI tract mainly by increasing mucosal protection and/or by reducing gastric acid. These drugs are largely used in conditions like peptic ulcers and include sucralfate, colloidal bismuth subcitrate and rebamipide.

Sucralfate

Sucralfate is a basic aluminium salt of sucrose octasulphate and has a primary unit that may be repeated as $C_{12}H_6O_{11}[SO_3^-Al_2(OH)_5^+]_8.nH_2O$. It occurs as a white, amorphous powder that is practically insoluble in alcohol or water. Sucralfate is structurally related to heparin, but it does not possess any appreciable anticoagulant activity. It is also structurally related to sucrose, but it is not utilised as a sugar by the body. It is a drug of its own kind that binds to and protects the ulcerated site from acid, bile and pepsin activity.

Mechanism of action and pharmacological effects

In the acidic environment of stomach, sucralfate breaks down into sucrose sulphate and its aluminium salt. The sucrose polymerises at pH <4.0 by cross linking of molecules, assuming a sticky gel like consistency. Then negatively charged sulphate groups binds electrostatically to positively charged proteins in ulcerated and necrotic tissues, which promotes faster healing and protects the ulcer from further damage caused by acid and pepsin. In addition, it prevents back diffusion of hydrogen ions and adsorbs both pepsin and bile acids. Binding occurs in the base of ulcer craters and is greater in duodenal compared to gastric ulcers. Dietary proteins adsorb to its surface, thus forming another cytoprotective layer. The affinity of sucralfate for abnormal and damaged tissue is five times greater than for normal mucosa. Antacid and food do not appear to affect the integrity of the adherent gel.

Some other effects have been demonstrated for sucralfate which include stimulated prostaglandin release from gastric mucosa, increased mucus viscosity, increased mucus output, increased bicarbonate secretion in stomach, and increased prostaglandin dependent blood flow and mucosal repair. Sucralfate appears to be the stimulus for potential formation of local mediators such as PGs, and possibly sulphydryl ions or other oxygen radical scavengers which protect gastric mucosa. Sucralfate also binds bile salts, which are implicated in the pathogenesis of gastric ulcers. In addition to binding and protecting cells, the polymerised sucrose prevents exudation of protein and electrolytes into the gastric lumen. Sucralfate has no acid neutralising action, but it has been found to delay gastric emptying thus prolonging its own stay in the stomach.

Pharmacokinetics

Sucralfate is minimally absorbed (3-5%) following oral administration and is excreted largely unchanged in urine in 48 hours. The main portion of drug is converted to sucrose sulphate in the gut by reacting with HCl and is excreted in the faeces within 48 hours. The duration of action (binding to ulcer site) may persist up to 6 hours after oral dosing.

Side effects/Adverse effects

Adverse effects are uncommon with sucralfate therapy. Sucralfate is recognised to be the safest drug available for the treatment of gastroduodenal ulcers. Constipation due to Al^{+3} is the most prominent adverse effects reported in humans (2%) and dogs taking the drug. Laboratory animals receiving up to 12 g/kg of sucralfate orally demonstrated no incidence of mortality. Effects on plasma Al^{+++} and phosphate metabolism are similar to those described for aluminium hydroxide.

Drug interactions

Sucralfate can adsorb and thereby reduce bioavailability of a number of drugs including tetracyclines, phenytoin, digoxin, cimetidine, ketoconazole and fluoroquinolones. Antacids should not be given along with sucralfate because its polymerisation is dependent on acid pH. Interactions may be minimised by administration of other medications 2 hours prior to administration of sucralfate.

Clinical uses

Sucralfate has been used in the treatment of oral, oesophageal, gastric and duodenal ulcers. It has also been employed to prevent drug-induced (e.g. NSAIDs) gastric erosions. It reduces but does not prevent the number of GI ulcers associated with NSAIDs in horses. It is also effective for treatment of acid-induced oesophagitis, although antisecretory drugs such as omeprazole or H_2-receptor antagonists are superior. Studies in human and cats have supported use of sucralfate for reflux oesophagitis. Sucralfate should be given at least 1 hour before the meal.

Dose

Dogs	:	40 mg/kg, PO, 3 times daily.
Cats	:	250 mg (total), PO, 2 to 3 times daily.
Foals	:	2 mg/kg, PO, 3 times daily.

Colloidal bismuth subcitrate

Colloidal bismuth subcitrate (CBS) is a colloidal bismuth compound (tripotassium dicitratobismuthate). After ingestion of CBS, the bismuth derivatives formed in the acid environment of stomach bind strongly to the proteins in ulcerated tissue to form a protective layer which shields it from aggressive factors and allows it to heal. In addition CBS brings about an increase in local prostaglandins level, which stimulates the production of bicarbonate and mucin thereby also protecting the stomach. It is reported to be effective against *Helicobacter pylori*, a G –ve bacillus that is closely associated with gastritis and ulcer recurrence. Healing of the underlying gastritis by eradication of *H. pylori* prevents ulcer relapse.

Although CBS is a locally active agent, minute quantities of bismuth derived from it (less than 0.2% of the dose) are absorbed during therapy. The major portion of ingested bismuth is excreted with the faeces. Absorbed bismuth is excreted in urine and its blood level declines rapidly after completion of therapy. Colloidal bismuth subcitrate is indicated for treatment of duodenal and gastric ulceration. It may also be used in conjunction with antibiotic therapy for treatment of non-ulcer dyspepsia due to *H. pylori* associated gastritis. Colloidal bismuth subcitrate is not an antacid, but heals about 60% ulcers at 4 weeks and 80-90% at 8 weeks in human beings. Eradication of the bacillus reduces incidence for relapse of ulceration by about 90%. Use of CBS is contraindicated in severe renal impairment.

Rebamipide

Rebamipide is an amino acid derivative of 2(1H)-quinolinone. It possesses gastric protective action and acts by enhancing

mucosal defence, scavenging free radicals and temporarily activating genes encoding cyclooxygenase-2. Rebamipide has been recommended for the GI mucosal protection, healing of gastroduodenal ulcers and treatment of gastritis. It also protects the gastric mucosa against NSAIDs induced adverse effects.

Anti-Helicobacter drugs

The use of antibacterial drugs and bismuth in humans has been aimed at eradicating the bacteria *Helicobacter pylori*. The current literature suggests that *H. pylori* is the most common and important cause of gastritis in humans. In addition, the bacillus has been associated with duodenal ulcers, gastric ulcers, non-ulcer dyspepsia and gastric cancer. Suppression or eradication of *H. pylori* infection significantly increases the healing rate and reduces the rate of ulcer relapse.

The drugs most commonly used to eradicate *H. pylori* infection are combination of therapy with bismuth and metronidazole or amoxicillin, or all three drugs. *Helicobacter pylori* has not been apparently associated with gastritis or GI ulcers in small animals. An organism similar to *H. pylori* has been isolated in cats, but its importance is not clear.

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CHAPTER 43 DRUGS AFFECTING GASTROINTESTINAL MOTILITY

- Prokinetics
  - Mediators of gastric motility
  - Classification
  - Dopamine (D_2)-receptor antagonists serotonin
  - Serotonin (5-HT_4)-receptor agonists
  - Motilin-like drugs
  - Histamine (H_2)-receptor antagonists
  - Prostanoids
- Drugs affecting intestinal motility
  - Physiology of intestinal regulation of water and electrolytes
    - Absorption of water and electrolytes
    - Secretion of water and electrolytes
  - Laxatives and cathartics
    - Classification
    - Lubricant laxatives
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    - Simple bulk purgatives
    - Osmotic purgatives
      - Inorganic salts
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    - Irritant/stimulant purgatives
      - Mechanism of action
      - Direct irritant purgatives
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        - Inorganic compounds
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        - Vegetable oils
        - Anthraquinone/ anthracene purgatives
          - Vegetable anthraquinone purgatives
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      - Prostanoids
      - Enema/Enemata
- Antidiarrhoeal drugs
  - Diarrhoea
  - Classification
  - Non-specific antidiarrhoeal drugs
  - Gastrointestinal protectants and adsorbents
  - Antimotility drugs
  - Antisecretory and anti-inflammatory agents
    - Salicylic acid derivatives
    - Anticholinergics
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  - Replenishment of fluid and electrolyte loss
    - Oral rehydration solutions
    - Intravenous rehydration
  - Maintenance of nutrition

PROKINETICS

Prokinetics (Gastroprokinetics, gastrokinetics) are agents which increase motility of a segment of the gastrointestinal tract and thereby augment transit of intraluminal contents through that area. These are mainly used in the gastric/gastroduodenal motility disorders. A gastric motility disorder is usually characterised by history of chronic vomiting, which may or may not be associated with feeding. Other signs of a gastric motility disorder may include anorexia, indigestion, belching, post-prandial discomfort, gastro-oesophageal reflux and weight loss. Repeated reflux of gastric contents into the lower oesophagus has been associated with oesophagitis and ulcers. In such conditions, prokinetic agents stimulate contractions in the gastropyloroduodenal area and accelerate gastric emptying. However, all agents which stimulate gastric contractions may not stimulate rate of gastric emptying (e.g. bethanechol)

Mediators of Gastric Motility

The motility of true stomach is regulated by a complex interaction of various neurotransmitters and modulators. Gastric motility is stimulated by cholinergic neurons and inhibited by adrenergic neurons. It is also influenced by enteric nervous system in which dopamine and serotonin play a role. Dopamine through D_2 receptors usually inhibits fundus movement, reduces amplitude of contractions, delays gastric emptying (when food is present in stomach) and mediates gastric dilatation/lower oesophageal sphincter relaxation through inhibition of acetylcholine release. Serotonin, on the other hand, enhances acetylcholine release from the myenteric plexus through 5-HT_4 receptors. In addition to these mediators, the gastrointestinal peptide motilin stimulates gastric emptying and postprandial gastric contraction via specific motilin receptors present in the antrum and duodenum.

Classification

Prokinetic agents belong to distinct chemical classes having variable mechanisms of action. Broadly these agents have been divided into following groups:

I. Dopamine (D_2)-receptor antagonists
   e.g. metoclopramide, domperidone, bromopride, alizapride and clebopride.
II. Serotonin (5-HT_4)-receptor agonists
   e.g. cisapride, tegaserod, prucalopride, mosapride and cinitapride.
III. Motilin-like drugs
   e.g. erythromycin.
IV. Histamine (H_2)-receptor antagonists
   e.g. ranitidine and nizatidine.
V. Prostanoids
   e.g. misoprostol.

I. Dopamine (D_2)-Receptor Antagonists

Metoclopramide

Metoclopramide is a substituted benzamide that is used as gastric prokinetic and anti-emetic agent. It is structurally related to procainamide (derivative of para-amino benzoic acid), but lacks significant local anaesthetic action. Metoclopramide is primarily used to treat nausea and vomiting, and to facilitate gastric emptying in patients with gastroparesis. It is also a primary treatment for migraine headaches. Metoclopramide is photosensitive and must be stored in light resistant containers.

Mechanism of action

Metoclopramide is a dopamine D_2-receptor antagonist with central and peripheral activities. It is also a mixed 5-HT_3 receptor antagonist/ 5-HT_4 receptor agonist. The prokinetic effect is mediated through antagonism of dopamine D_2 receptor, agonism of 5-HT_4 receptors and cholinomimetic effects in the GI tract. Metoclopramide as a D_2-receptor antagonist enhances release of acetylcholine from myenteric neurons which augments peristalsis (Figure 43.1). Increased secretion of ACh from myenteric plexus neurons may also be mediated through 5-HT_4-receptor stimulation. Atropine and other antimuscarinic drugs can antagonise prokinetic effect of metoclopramide.

The antiemetic effect of metoclopramide is mediated through antagonism of dopaminergic D_2 receptors on CTZ and stomach (see also Chapter 44). At high dosages, metoclopramide also blocks 5-HT_3-receptors present on vagal afferent in GI tract and in the NTS/CTZ in CNS to produce antiemetic effects.

Pharmacological effects

The important pharmacological effects of metoclopramide are associated with the GI tract and CNS.

GI tract: In the GI tract, metoclopramide relaxes the pyloric sphincter, enhances and amplitude of gastric contractions, and increases peristalsis of the duodenum and jejunum. It thus facilitates gastric emptying with decreased transit time of ingesta in the stomach and proximal small intestine. It also increases tone of the oesophageal sphincter and prevent gastroesophageal reflux. Metoclopramide increases motility of gastrointestinal tract without affecting the gastric, pancreatic or biliary secretions. There is little or no effect on colon motility. The peripheral prokinetic effect of metoclopramide also gives its local anti-emetic effect. In normal dogs, gastric emptying time is not significantly shortened by metoclopramide.

Central nervous system: In the CNS, metoclopramide apparently antagonises dopamine at the receptor sites. This action is responsible for its central antiemetic, sedative, extrapyramidal and prolactin secretion stimulating effects. The effects of metoclopramide on emesis are discussed in Chapter 44.

Pharmacokinetics

Metoclopramide is absorbed well after oral administration but it undergoes significant first-pass metabolism with a bioavailability of 50-70%. The drug is rapidly distributed in body and enters CNS. Metoclopramide is only weakly bound to 15-20% of plasma proteins. The drug also crosses the placenta and enters the milk. It is metabolised to 75-80% to glucuronide and sulphate conjugates. The unchanged drug (20-25%) and metabolites are excreted in urine (mainly) and faeces (5%). The half-life of metoclopramide in dogs has been reported to be about 90 minutes.

Side effects/Adverse effects

Side effects of metoclopramide in domestic animals are uncommon, but extrapyramidal effects such as behaviour changes and hypersensitivity may occur, particularly with large IV doses. Cats may exhibit signs of frenzied behaviour or disorientation. In rare instances, it may cause gastric spasm. In adult horses, IV metoclopramide may produce severe CNS effects with behavioural changes and alternating periods of sedation and excitement. In very high doses, metoclopramide may produce ataxia, sedation, agitation, nausea and vomiting in different species of animals. In human beings, long-term use can cause Parkinsonism, galactorrhoea and gynaecomastia. Drug-induced movement disorders are quite common with long-term use.

Treatment: There is no specific antidote to metoclopramide intoxication. In recent ingestion, stomach should be emptied using standard measures. First generation antihistaminic agents which enter CNS and possess anticholinergic effects (e.g. diphenhydramine) may be helpful in controlling extrapyramidal effects.

Contraindications and precautions

Metoclopramide is contraindicated in patients with GI haemorrhage, obstruction or perforation. It is contraindicated in patients with seizures disorders (epilepsy) and in animals receiving narcoleptics. Use in horses should be done cautiously as it may lead to dangerous behaviour changes.

Drug interactions

Metoclopramide should not be used along with atropine and opioid analgesics because they antagonise the prokinetic effect of metoclopramide. Phenothiazines (e.g. chlorpromazine) and butyrophenones (e.g. droperidol) may potentiate the extrapyramidal effect of metoclopramide and CNS depressants like tranquillizers and sedatives may enhance the CNS depression produced by metoclopramide. Metoclopramide may decrease bioavailability of drugs which are absorbed in stomach (e.g. digoxin) but it enhances bioavailability of drugs absorbing from small intestine.

Clinical uses

Metoclopramide is widely used in human and veterinary medicine for prokinetic and antiemetic effects. As a prokinetic, it is useful in indigestion, belching and gastroesophageal reflux disease (GERD/GORD). The prokinetic effects make

metoclopramide useful in the treatment of gastric stasis (e.g. after gastric surgery or diabetic gastroparesis). In ruminants, it may be used in ruminal atony and oesophageal reflux. As an anti-emetic, metoclopramide is considered very useful in treatment of nausea and vomiting associated with conditions such as emetogenic drugs, uremia, radiation sickness, malignancy, labour and infection (see also Chapter 44). Due to its dopamine receptor inhibitory action, metoclopramide is also considered useful to stimulate lactation. In human medicine, it is also used in the treatment for migraine headache.

Dose

As prokinetic agent

Dogs & cats	:	0.2 – 0.5 mg/kg, PO, SC or IV, 3 times daily. It is often given 30 minutes before meals.
	:	0.01 – 0.02 mg/kg, continuous IV infusion (total 1 – 2 mg/kg/day).
	:	0.2 mg/kg, PO, 3 times daily (with cimetidine or ranitidine, and high protein- low fat diet).
Horses	:	0.02 – 0.1 mg/kg, IM or IV, 3 to 4 times daily.
Cattle	:	0.5 – 1 mg/kg, IV followed by IM injection (same dose) 2 times daily (maximum 2 IM doses).

Domperidone

Domperidone is a derivative of benzimidazole that possesses both prokinetic and antiemetic properties. It is chemically related to haloperidol, but pharmacologically related to metoclopramide.

Mechanism of action

Domperidone is a dopamine antagonist. It blocks D_2 receptors in the stomach and initiates prokinetic effect. However, unlike metoclopramide, its prokinetic action is not blocked by atropine. Inhibition of D_2 and D_3 dopamine receptors, which are found in the chemoreceptor trigger zone, produces anti-emetic effects.

Pharmacological effects

Pharmacological effects of domperidone resemble metoclopramide, but its antiemetic and prokinetic effects are lesser than metoclopramide. It has negligible effects on colonic motility. Although it does not readily cross blood-brain barrier, domperidone still can exert effects on those parts of the central nervous system which do not possess this barrier, such as those regulating emesis (act on CTZ), temperature and prolactin release. However, unlike other D_2-receptor antagonists, extrapyramidal effects are rare.

Pharmacokinetics

Domperidone appears to be rapidly absorbed after oral administration, but its bioavailability is only about 15% in man due to first-pass metabolism. Domperidone cannot cross the blood-brain barrier in sufficient concentration. It is extensively metabolised in the body and metabolites are excreted mainly in urine.

Side effects/Adverse effects

Side/Adverse effects of domperidone are much lesser than metoclopramide because it does not cross the blood brain barrier as readily as metoclopramide. Important side effects include dry mouth, loose stools, rashes and galactorrhoea. On rapid IV injection, domperidone may result in cardiac arrhythmias.

Contraindications and Drug interactions

Domperidone should not be used in animals with GI obstruction. It should not be administered along with antacids as it needs acid medium for absorption after oral administration.

Clinical uses

Domperidone is used as a prokinetic agent in conditions like gastroparesis and gastroesophageal reflux. It is also used as an anti-emetic agent in the treatment of various vomiting disorders either alone or in combination with metoclopramide, antimuscarinic agents (e.g. cyclizine) or $5HT_3$ receptor antagonists (e.g. granisetron). When nausea and gastric retention are chronic and debilitating, domperidone may be a useful agent for the long-term treatment. Domperidone is also used to treat fescue toxicity and periparturient agalactia in horses. A commercial domperidone oral gel preparation (110 mg/ml) is available for use in horses.

Dose

Dogs	:	0.1 – 0.5 mg/kg, IM.
	:	0.5 – 1 mg/kg, PO.
Horses	:	1.1 mg/kg, PO, once daily starting 10 days before the schedule foaling date and continued until foaling. If case of inadequate milk production after foaling, the drug should be continued for 5 additional days.

Bromopride

Bromopride is a substituted benzamide and a close congener of metoclopramide. It is identical to metoclopramide except for the presence of a bromine atom where metoclopramide has a chlorine substituent. Similar to metoclopramide, bromopride is a dopamine antagonist with prokinetic and anti-emetic properties. It is mainly indicated in the treatment of nausea and vomiting and gastroesophageal reflux disease (GERD/GORD).

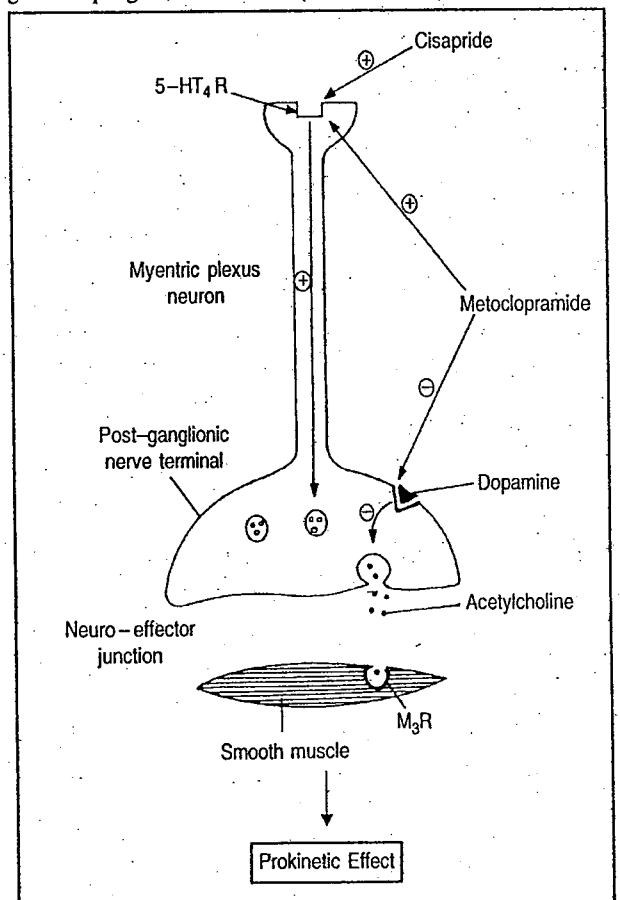


Fig. 43.1. Mechanism of action of prokinetic drugs (see text for detail). **Abbreviation :** 5-HT_4 R = 5-hydroxytryptamine - 4 receptor, M_3R = muscarinic - 3 receptor; + = stimulation; – = inhibition.

Other drugs

Alizapride and clebopride are two other prokinetic and antiemetic drugs available for use in human medicine. These drugs are structurally and pharmacologically related to metoclopramide and act by blocking dopamine receptors. They are used to treat functional gastrointestinal disorders, and nausea and vomiting.

II. Serotonin (5-HT_4)-Receptor Agonists

Cisapride

Cisapride is a substituted benzamide that is used as a prokinetic agent. It is structurally related to procainamide, but has no local anaesthetic action. Cisapride is used mainly in animals to treat gastric hypomotility disorders.

Mechanism of action

Cisapride is a 5-HT_4-receptors agonist. The 5-HT_4 receptors are present on the enteric post-ganglionic cholinergic neurons and help to enhance release of acetylcholine from the neurons in the myenteric plexus (Figure 43.1). Cisapride also has 5-HT_1/5-HT_3 antagonistic effects on enteric cholinergic neurons. It has no or negligible antidopaminergic effect. It does not act on CTZ and has no central depressant effect. The efficacy of cisapride in the treatment of disorders of gastric hypomotility appears to equal that of metoclopramide and domperidone, however, its usefulness as antiemetic drug is less.

Pharmacological effects

The effect of cisapride on the motility of stomach and small intestine closely resemble that of metoclopramide; however, unlike metoclopramide, it also stimulates colonic motility and may cause diarrhoea. Cisapride as prokinetic increases lower oesophageal peristalsis and sphincter pressure and accelerates gastric emptying. In this regard, cisapride appears to be superior to metoclopramide and domperidone. Its activity as prokinetic is about 8 times more than metoclopramide. It exerts a prokinetic effect from the lower oesophageal sphincter up to and including colon. In contrast to metoclopramide that stimulates the stomach and proximal intestine and primarily consists of enhancing antropyloroduodenal contractions, cisapride increases lower oesophageal sphincter tone, speeds up gastric emptying of solid and liquids, decreases small intestine and large intestine transit times, enhances coordination of antroduodenal function, and increases antral, duodenal and colonic motility. Clinically, it succeeds in stimulating gastric peristalsis in animals when metoclopramide fails. It principally affects smooth muscles (not skeletal muscles), so has little effect on oesophageal function. Cisapride does not possess antidopaminergic effect, so it does not influence the concentration of prolactin in plasma or cause extrapyramidal symptoms.

Pharmacokinetics

The detailed pharmacokinetic data on cisapride in animals are lacking. In human beings, cisapride is rapidly absorbed after oral administration and is highly bound to plasma proteins. It is extensively metabolised by hepatic N-dealkylation and hydroxylation and excreted in the faeces.

Side effects/Adverse effects

The main adverse effect related to cisapride is diarrhoea. In high doses in dogs, cisapride may produce diarrhoea, abdominal cramps, muscular tremors, fasciculation, ataxia, incoordination, hyperthermia, dyspnoea, disorientation and/or hyperactivity. Cisapride produces serious cardiac effects in humans including QT interval prolongation and slowing of cardiac repolarisation resulting in *torsades de pointes* and fatal ventricular arrhythmias. Although similar effects have been reported in dogs and cats, the adverse effects on heart are not serious. Unlike metoclopramide and domperidone, cisapride does not produce side effects which result from blockade of dopamine receptors.

Contraindications and precautions

Cisapride is contraindicated in animals in which enhanced gastrointestinal motility could be harmful (e.g. perforation, obstruction and GI haemorrhage).

Drug interactions

The facilitation of gastric emptying by cisapride could affect the absorption of other orally administered drugs. Oral drugs with narrow therapeutic index (e.g. digoxin and anticonvulsants) need serum levels monitored closely when used with cisapride due to their enhanced absorption. Concurrent administration of cimetidine (not ranitidine) may lead to increased plasma cisapride concentrations as cimetidine inhibits cytochrome P-450 enzyme system. Simultaneous use of ketoconazole (or related drugs) or macrolide antibiotics with cisapride may enhance serious cardiac arrhythmia. Concurrent administration of anticholinergic drugs (e.g. atropine) may diminish effects of cisapride.

Clinical uses

Cisapride is therapeutically useful in conditions like gastrointestinal reflux, delayed gastric emptying, colonic hypomotility in feline megacolon and chronic constipation, especially in cats. When used for chronic constipation, it is beneficial to remove excessively large or firm faeces before giving cisapride. Cisapride is also useful in gastroparesis (gastric relaxation) seen after surgery or after an episode of gastric dilation/volvulus. Cisapride has shown to increase gut motility in chronic grass sickness in horses and is useful in post-operative ileus in equines. It is not/less used in human medicine because of reports of long QT syndrome which predisposes to arrhythmias.

Dose

Dogs	:	0.1 – 0.5 mg/kg, PO, 2 to 3 times daily. Dose may be increased to 0.5 – 1 mg/kg, PO, 2 to 3 times in delayed gastric emptying.
Cats	:	1 – 1.5 mg/kg, PO, 2 to 3 times daily.
Horses	:	0.5 – 0.8 mg/kg, PO, 3 times daily for 7 days.

Tegaserod

Tegaserod is a non-benzamide colonic prokinetic drug. It acts as a potent partial agonist at 5-HT_4 receptor and a weak agonist at 5-HT_{1D} receptors. It primarily produces prokinetic effects in the intestine, particularly colon, causing enhanced intestinal transit and colonic propulsion. Therefore, it may be useful in various intestinal disorders including constipation, intestinal ileus and pseudo-obstruction. It does not produce prokinetic effect on the stomach, so is not considered useful as cisapride in the treatment of delayed gastric emptying. Tegaserod has recently been removed from the market due to suspected increased risks of heart attack or stroke in human patients.

Dose

Dogs	:	0.05 – 0.1 mg/kg, PO or IV, 2 times daily.

Prucalopride

Prucalopride is a benzamide drug with enterokinetic activities. Prucalopride stimulates colonic motility via serotonin 5-HT_4 receptor activation, which provides the main propulsive force for defecation. Unlike cisapride and tegaserod, prucalopride is highly selective (>150 fold) for serotonin 5-HT_4 receptor, with no action on other types of receptors. This appears to account for lesser adverse effects seen on the cardiovascular system. The most frequently reported side effects in human beings are headache and gastrointestinal symptoms (abdominal pain, nausea or diarrhoea). Prucalopride is approved for the symptomatic treatment of chronic constipation. It also seems to stimulate gastric emptying in dogs and increases defecation frequency in cats.

Other drugs

Mosapride and cinitapride are two other gastroprokinetic agents of the benzamide class. Mosapride acts as a selective 5-HT_4 agonist, whereas cinitapride acts as an agonist of the 5-HT_1 and 5-HT_4 receptors and as an antagonist of the 5-HT_2 receptors. Their pharmacological effects, side/adverse effects and clinical uses appear to be similar to those of cisapride.

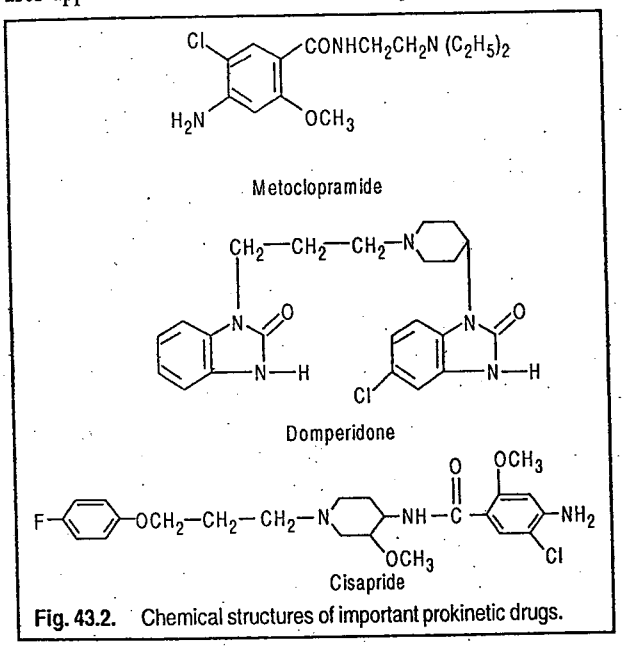


Fig. 43.2. Chemical structures of important prokinetic drugs.

III. Motilin-Like Drugs

Erythromycin

Erythromycin is an orally effective macrolide antibiotic. At low antimicrobially ineffective doses, erythromycin stimulates motility in the proximal gastrointestinal tract including lower oesophageal sphincter, stomach and small intestine. Erythromycin stimulates gastrointestinal motility by means of direct motilin-receptor activation (in cats) and indirect cholinergic and neurokinin activation (in dogs). Erythromycin, however, accelerates gastric emptying of solid food more rapidly so that more untriturated food (food particles > 0.5 mm) passes into small intestine. This may lead to inadequate digestion and absorption of nutrients and passing of more undigested food particle in faeces. If large particles of food in the small intestine cause intestinal distress, use of this prokinetic drug may not lead to an improvement and could actually increase the symptoms, despite more rapid gastric emptying.

Erythromycin may be used as a prokinetic agent to accelerate gastric emptying, to increase lower oesophageal sphincter pressure and to facilitate intestinal transit in patients with small intestinal motility disorders. Oral use of erythromycin is also recommended when the oral cisapride fails to improve gastric emptying. No serious drug interactions have been reported with erythromycin in animals. The prokinetic dosage of erythromycin is much lower than its antimicrobial dosage (see Chapter 63).

Dose

As a prokinetic agent

Dogs & cats : 0.5 – 1 mg/kg, PO, 3 times daily.

IV. Histamine (H_2)-Receptor Antagonists

Ranitidine and Nizatidine

Ranitidine and nizatidine are reversible competitive H_2-histamine receptor antagonists with gastric antisecretory properties. Ranitidine and nizatidine are also used as gastrointestinal prokinetic agents. They stimulate gastrointestinal motility at gastric antisecretory dosages by inhibiting acetylcholinesterase enzyme activity, which in turn increases amount of acetylcholine available to bind to the smooth muscle muscarinic receptors. The prokinetic effect of these two drugs may be comparable to that of cisapride. Other members of the H_2-receptor antagonist group like cimetidine and famotidine apparently have no effect on the gastrointestinal motility.

Ranitidine and nizatidine are considered useful in the treatment of gastric emptying disorders and constipation as well as gastric ulcers. They appear to have wide margin of safety. Concurrent use of anticholinergic compounds like atropine would be expected to block the beneficial effect of ranitidine and nizatidine.

Dose

As prokinetic agent

Ranitidine

Dogs and cats : 1 –2 mg/kg, PO, 2 times daily.

Nizatidine

Dogs and cats : 2.5 – 5 mg/kg, PO, once daily.

V. Prostanoids

Misoprostol

Misoprostol is a prostaglandin E_1 analogue (see Chapter 29). In addition to acting as a cytoprotective and antiulcer agent, misoprostol is reported to increase colonic propulsive activity. Due to its limited toxicity, misoprostol may be considered useful in dogs and cats with severe refractory constipation.

Dose

As prokinetic

Dogs : 0.002 – 0.005 mg/kg, PO, 3 to 4 times daily.

DRUGS AFFECTING INTESTINAL MOTILITY

Intestine is the part of the gastrointestinal tract where the vast majority of digestion and absorption of food takes place. Alterations in the absorptive, secretory and motor functions of the intestine adversely affect the digestion and absorption of food and can lead to either diarrhoea or constipation. Since the extent of absorption generally parallels transit time, altered motility also contributes to diarrhoea or constipation.

PHYSIOLOGY OF INTESTINAL REGULATION OF WATER AND ELECTROLYTES

Water and electrolytes are absorbed as well as secreted in the intestine. Normally, the magnitude of intestinal fluid and ion absorption greatly exceeds that of intestinal secretion, with more than 98 percent of all fluid entering the intestine (both ingested and intestinal secretions) being absorbed. Specialised transport systems exist in the apical (luminal) and basolateral membranes, which allow absorption and secretion of water and electrolytes. Intestinal villi, the tiny, finger-like projections which protrude from the epithelial lining of intestinal wall are mainly responsible for intestinal absorption of electrolyte and nutrients. The crypt cells located at the base of villi have a secretory function.

Absorption of Water and Electrolytes

Water

A normal person or animal of similar size takes in roughly 1 to 2 litres of dietary fluid every day. Another 6 to 7 litre of fluid is received by the small intestine daily as secretions from salivary glands, stomach, pancreas, liver and the small intestine itself. This large amount of water is absorbed from the intestinal wall, which is freely permeable to water allowing it to move in either direction. As electrolytes and other soluble nutrients are absorbed, water is drawn isosmotically from lumen to intestinal capillaries by either the paracellular (around the cell) or the transcellular (through the cells) diffusion routes.

Sodium

The absorption of sodium in intestine occurs by at least three distinct mechanisms viz. simple diffusion, sodium co-transport and Na^+–H^+ exchanger. Absorption of sodium across the apical membrane of jejunum (mainly) occurs by passive diffusion. Sodium co-transport protein (symport) carries two sodium ions along with one molecule of glucose across the apical membrane from lumen into intracellular fluid. The Na^+–H^+ exchanger (antiport) allows exchange of intracellular H^+ with luminal Na^+ (Figure 43.3). As the H^+ generated by carbonic acid is exchanged for Na^+, the intracellular HCO_3^- concentration builds up that is exchanged for luminal Cl^- via the Cl^-–HCO_3^- exchanger. This mechanism is most active in the ileum and colon where Na^+ concentration is relatively low. Because of close connection between Na^+ and Cl^- absorption of these pathways, the mechanism is often called coupled sodium-chloride transport. Sodium entering the enterocyte is transported across the basolateral membrane via the Na^+-K^+-ATPase pump, which keeps the intracellular Na^+ concentration low and is the main driving force for various Na^+ absorption mechanisms.

Chloride

Absorption of chloride in the intestine also involves three major mechanisms viz. paracellular absorption, coupled sodium-chloride transport and chloride-bicarbonate exchange. In the paracellular transport, chloride from the gut lumen passes directly into the lateral spaces (spaces between cells) through tight junctions between cells, which are permeable to only water and small electrolytes. This occurs generally in response to positively charged Na^+ ions coming to lateral spaces through the sodium co-transport. The paracellular absorption provides a major mechanism for absorption of chloride ion while maintaining electrical neutrality. The coupled sodium-chloride absorption mechanism is the same, as discussed previously in relation to sodium. The absorption of chloride through chloride–bicarbonate exchange (Figure 43.3) occurs especially in areas where HCO_3^- secretion into intestinal lumen is important and results in increased lumen pH. Bicarbonate ion entering the intestine reacts with HCl (to form water, carbon dioxide and sodium chloride), reabsorbed

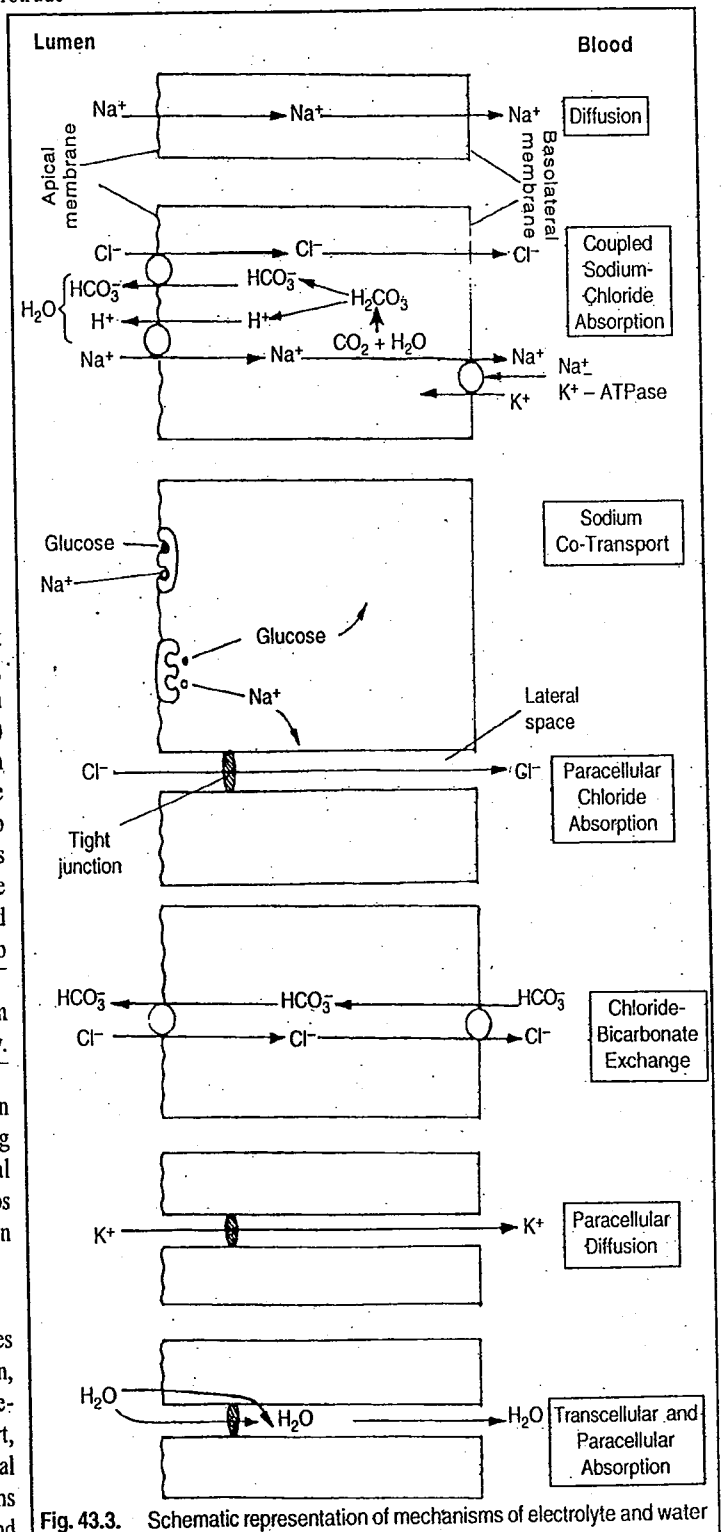


Fig. 43.3. Schematic representation of mechanisms of electrolyte and water absorption.

by ion-exchange mechanism primarily in the ileum and colon or is neutralised by H^+ secreted in lumen in exchange for sodium. Within cell, the bicarbonate ion is regenerated from water and CO_2, which is either secreted into the lumen or electrically balanced by sodium ion and absorbed as sodium bicarbonate. To maintain the acid-base balance in body, bicarbonate ion secreted by several digestive glands is recovered in efficient way.

Potassium

Potassium is absorbed by simple diffusion through paracellular route mainly in the lower intestine. Potassium absorption is directly coupled to water absorption because water absorption in the upper intestine increases concentration of K^+ in the lower intestine, which creates a favourable diffusion gradient for potassium. Therefore in conditions like diarrhoea in which net absorption of water is impaired, potassium absorption is also impaired because potassium in the gut lumen is diluted and a concentration gradient favourable for passive diffusion of K^+ never develops. In addition to potassium diffusion, it is suggested that an H^+-K^+-ATPase exists in distal colon, which recovers last remaining K^+ from the colonic ingesta.

Secretion of Water and Electrolytes

In contrast to the absorptive function of villous cells in intestine, the crypt cells have a secretory function. In the crypt cells, the coupled sodium-chloride mechanism is present on the basolateral membrane, which pumps both sodium and chloride into the crypt enterocytes from lateral spaces. From enterocytes, sodium is quickly pumped out by the Na^+-K^+-ATPase (sodium pump); however, chloride is trapped inside and reaches high intracellular concentration. High intracellular chloride causes chloride channels in the apical membranes to open resulting in movement of chloride into the lumen. This creates an electrical attraction for sodium ions, which too moves into the lumen from the lateral spaces through the paracellular route. Water follows sodium and chloride osmotically. In addition to electrolytes, digestion of food creates many osmotically active molecules from a big precursor molecule (e.g. formation of di- and tri-saccharides from single starch molecule). These osmotically active molecules draw water into the intestinal lumen. However, as digestion proceeds, osmotically active molecules are absorbed and water follows them osmotically back into the blood.

Intracellular cyclic nucleotides are important regulators of absorption and secretory processes. Stimuli enhancing cAMP and cGMP cause net loss of sodium chloride and water, both by inhibiting NaCl absorption in villous cells and by promoting anion secretion (Na^+ accompanies) in crypt cells. It is seen that when cAMP concentration increases, chloride gates open and water and electrolyte secretion is stimulated. Colonic function is regulated by many hormones and neurotransmitters, which influence absorption and secretion of water and electrolytes. These include somatostatin, opioids, antidiuretic hormone, aldosterone, and dopaminergic and adrenergic agonists which enhance absorption or inhibit secretion, and vasoactive intestinal peptide (VIP), prostaglandins and cholinergic agonists which enhance secretion. In some systems, these agents act to inhibit (e.g. somatostatin and opioid peptides) or to stimulate (e.g. prostaglandin E_2 and VIP) adenylate cyclase activity.

LAXATIVES AND CATHARTICS

Laxatives and cathartics are agents which promote defecation either by increasing the frequency of defecation or by enhancing faecal volume or consistency. Laxatives (also called aperients) have milder action and promote elimination of soft but formed stool, whereas cathartics (also called purgatives) are stronger in action and tend to produce a more fluid evacuation. Many drugs in low doses act as laxative and in higher doses as purgatives, but some drugs are only laxatives without inducing catharsis even in very high doses. Laxative and cathartics are commonly used in constipation.

Constipation

Constipation is defined as infrequent or absent passage of faeces and is characterised by straining to defecate and by retention of hard dry faeces in the colon and rectum. Constipation is not a disease but a symptom that may result from a broad variety of underlying causes. Constipation may result from a variety of agents which prolong the faecal transit time and allow continued absorption of water, producing a dry and hard faecal mass. Several pharmacological agents are available which promote evacuation of GI tract over several days or within few hours. However, as the constipation is a symptom and not a disease, effective treatment of constipation may require first determining the cause. As the bulk, softness and hydration of faeces are very dependent on the fibre and water content of diet, the sufficient dietary fibre and water intake are mainstays in any regimen for the treatment of constipation. The specific therapeutic plan of constipation is determined mainly by severity of constipation and underlying cause and also adverse effects of drugs.

Classification

Laxatives and cathartics are usually classified by their general mode of action.

I. Lubricant laxatives
   e.g. liquid paraffin, white and yellow soft paraffin.
II. Surfactant laxatives/Stool softeners
   e.g. docusates (sodium calcium, potassium) and poloxamers.
III. Simple bulk purgatives
   e.g. bran, agar, ispaghula, sterculia, methylcellulose, carboxymethylcellulose and polycarbophils.
IV. Osmotic purgatives
   1. Inorganic salts/Saline osmotic purgatives
      e.g. magnesium sulphate, magnesium hydroxide and sodium sulphate.
   2. Carbohydrate osmotic purgatives
      e.g. lactulose, lactitol, glycerine, sorbitol, mannitol and macrogol (polyethylene glycol).
V. Irritant/Stimulant purgatives
   1. Direct irritant purgatives
   a. Diphenylmethanes
      e.g. bisacodyl and phenolphthalein.
   b. Inorganic compounds
      e.g. mercurous chloride and sulphur.
   2. Indirect irritant purgatives
   a. Vegetable oils
      e.g. castor oil and linseed oil.

b. Anthraquinone/Anthracene purgatives
   e.g. aloes, cascara sagrada, senna, rhubarb and dantron.
3. Drastic irritant purgatives
   e.g. jalap, podophyllum, croton oil and barium chloride.

VI. Neuromuscular purgatives
   e.g. carbachol, physostigmine and neostigmine.

VII. Peripheral opioid antagonists
   e.g. alvimopan and methylnaltrexone.

VIII. Prostanoids
   e.g. lubiprostone.

IX. Enemas
   e.g. soapy water, warm water and saline solution.

I. Lubricant Laxatives

Lubricant laxatives are usually long chain hydrocarbons which are extremely resistant to chemical and physiological reactions, particularly to the digestion. So once administered, they remain almost entirely unchanged and unabsorbed throughout the length of GI tract. Their oily and lubricant properties soften and lubricate the faecal mass, which in turn facilitate expulsion.

Liquid paraffin

Liquid paraffin (Mineral oil, liquid petroleum) is a mineral oil and a commonly employed lubricant laxative. It is a clear, viscous oil liquid, and a mixture of petroleum hydrocarbons that is obtained as a residue from petroleum distillation. Mineral oil is available in light and heavy grades, and can often be found in drug stores.

Mode of action and pharmacological effects

On oral administration, mineral oil has a demulcent action in the mouth, oesophagus and stomach. In large intestine, it acts as a lubricant laxative where it softens stools and lubricates hard scybala (hard rounded faecal masses) by coating them. As the mineral oil temporarily coats the intestines, it prevents the uptake of certain essential vitamins and nutrients. Liquid paraffin is also used as enema.

Side effects/Adverse effects

Although considered safe, a few adverse effects of mineral oil are seen, especially when it is used in overdosage or repeatedly for longer time.

a. Liquid paraffin may impair the digestion and absorption of nutrients, particularly the fat-soluble vitamins.
b. Liquid paraffin may cause lipid pneumonia as it easily trickles into the lungs while swallowing.
c. Small amount of liquid paraffin may get absorbed after prolonged use, which may produce granulomatous lesions in the intestinal wall and regional lymph node.
d. Prolonged use of liquid paraffin may decrease the intestinal responsiveness to local irritation as it coats the mucosa. This may lead to further slowing of intestinal movement and constipation.
e. Large doses or continuous use of liquid paraffin in pet animals may result in anal seepage/leakage of faeces, which may result in soiling of bedding, furniture and carpets. This may also mislead the owner that the animal continues to defecate.
f. In case of intestinal obstruction, liquid paraffin can readily bypass a partial obstruction and its presence in the anal area may lead to false conclusion.
g. Liquid paraffin may interfere with healing in the anorectal region.

Contraindications and precautions

Mineral oil is never to be used in conjunction with other stool softeners as it increases likelihood of harmful absorption of mineral oil into the body.

Clinical uses

Liquid paraffin is used in the treatment of simple impaction. However, it should be used only occasionally due to adverse effects. When liquid paraffin is administered to large animals, it should be mixed with ginger or mustard (except when given by stomach tube) in order to prevent aspiration into respiratory tract. It may be mixed with food or sugar for administration in dogs and cats. It is not effective for chronic or severe impactions.

Other medical uses of mineral oil may include its topical application as a suspending and levigating agent in sulphur or iodine-based ointments. It may also be used to alleviate mild eczema, particularly when the use of corticosteroid creams is not desirable. Mineral oil is also used in obstetrics to lubricate the birth canal and facilitate the delivery. Certain mineral oils are used in livestock vaccines, as an adjuvant to stimulate a cell-mediated immune response to the vaccinating agent.

Dose

Dogs	:	5 – 30 ml (total), PO. Dose may be adjusted according to the body size.
Cats	:	2 – 6 ml (total), PO.
Pigs	:	25 – 300 ml (total), PO.
Horses	:	250 – 1000 ml (total), PO.
Cattle	:	250 – 500 ml (total), PO.

White or Yellow soft paraffin

Soft paraffin (Petrolatum) is a mixture of various paraffin hydrocarbons, usually obtained by purifying the residue left when crude petroleum is distilled. Soft paraffin occurs as a semi-solid, white (bleached) or yellow, translucent, bland, odourless, tasteless, soft mass, which is free from acidity or alkalinity. White or yellow soft paraffin are occasionally used orally in small animals as lubricant laxatives. Externally, the white and yellow varieties are employed in the preparation of paraffin ointment. The pharmacological effects and adverse effects of white and yellow soft paraffin are similar to those of liquid paraffin. Soft paraffins are commonly known by several trade-names including Paraffin jelly, Petroleum jelly and Vaseline.

Dose

White soft paraffin

Dogs	:	5 – 15 ml, PO.
Cats	:	1 – 5 ml, PO.

II. Surfactant Laxatives

Surfactant laxatives (Stool softeners) are anionic or non-ionic surfactants. They act primarily as stool softeners or stool-wetting agents by allowing mixing of water, lipids and other faecal material (little mixing of aqueous intestinal contents and material occur with oily liquid paraffin). By altering intestinal permeability, they also increase net water and electrolyte secretions in the intestine.

Docusates

Docusate is an anionic surfactant, available in sodium (docusate sodium, dioctyl sodium sulphosuccinate, DSS, DOSS), calcium (docusate calcium, dioctyl calcium sulphosuccinate) and potassium (docusate potassium, dioctyl potassium sulphosuccinate) salts. Docusate sodium, the prototype for this group, occurs as white, wax-like plastic solid with a characteristic odour. It is less soluble in water but is freely soluble in alcohol and glycerine.

Mechanism of action and Pharmacological effects

Docusate sodium and other docusates are anionic surface acting agents with wetting and emulsifying properties. They reduce surface tension and allow water and fat to penetrate the ingesta and formed faeces, thereby softening the stool. The softer stools are easily passed without much straining. Some studies have also suggested that the effect of docusate may not necessarily be all due to its surfactant properties. Docusates have also shown to increase both ion secretion and fluid permeability from colonic mucosal cells into the colon lumen.

Pharmacokinetics

The detailed pharmacokinetics of docusates is not well known. It is believed that a considerable portion of docusate is absorbed from the small intestine and then excreted into the bile after undergoing extensive metabolism.

Side effects/Adverse effects

At normal doses, adverse effects are rarely seen with docusates. In some cases, diarrhoea, cramps, nausea and intestinal mucosal damage are possible. In high dosage, dehydration due to excessive secretory effect can occur. By detergent action, docusates can disrupt the mucosal barrier and enhance absorption of many non-absorbable drugs (e.g. liquid paraffin). Therefore, it should not be given with mineral oil or anthraquinone purgatives. Docusate is also excreted in the milk and can produce a laxative effect in the suckling young one. Docusate sodium is a strong irritant for eyes and lungs, and also a skin irritant.

Contraindications and precautions

Docusate is contraindicated in patients with severe abdominal pain or ileus. It is not suitable for the treatment of chronic constipation or obstipation (severe constipation due to obstruction of intestine).

Clinical uses

Docusates are mainly used as stool softeners in small animals when faeces are hard or dry. They are also used in anorectal conditions, when passing of firm faeces would be painful or detrimental. Overall the efficacy of the docusates is slight and their potential for toxicity is significant. Docusate is also used as an emulsifying, wetting and dispersing agent.

Dose

Docusate sodium

Dogs & cats	:	2 mg/kg, PO, 1 to 2 times daily.
	:	1 – 2 paediatric rectal suppository, 4 times daily. The drug should be discontinued, if no response occurs following second dose.
Horses & cattle	:	5 – 15 g (total), PO.

Docusate calcium

Dogs & cats	:	3 mg/kg, PO.
Horses & cattle	:	7 – 22 g (total), PO.

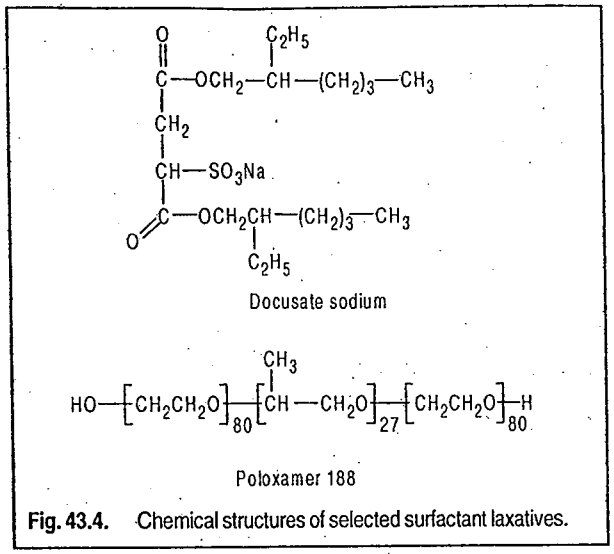


Fig. 43.4. Chemical structures of selected surfactant laxatives.

Poloxamers

Poloxamers are nonionic surfactants composed of a central hydrophobic chain of polyoxypropylene surrounded by two hydrophilic chains of polyoxyethylene. Because of their amphiphilic structure, the polymers possess surfactant properties. These polyoxyethylene-polyoxypropylene polymers were named "poloxamers" by Irving Schmolka, who invented and patented these materials in 1973. The lengths of the polymer blocks can be easily adjusted to make different types of poloxamers with different uses. The poloxamers are generally named with the letter "P" (for poloxamer) followed by three digits, the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content (e.g. P407 = Poloxamer with a polyoxypropylene molecular mass of 4,000 g/mol and a 70% polyoxyethylene content).

Poloxamer 188 (poloxalkol, poloxalene) is a water-soluble powder with actions similar to those of docusates. It has been used orally to break up assimilated faecal masses in the colon and rectum. It allows water and fats to penetrate, thus causing softening of the faeces. It is also used in the prevention of bloat in cattle. Poloxamers are also used to preferentially target cancer cells, due to differences in the membrane of cancerous cells when compared to normal cells. In this regard some poloxamers such as P85 are used to transport target genes to target cells and also to increase gene expression.

III. Simple Bulk Purgatives

Simple bulk laxatives/purgatives, also known as bulking agents, are hydrophilic substances which absorb water and increase bulk of intestinal ingesta. Various natural and semi-synthetic agents are used as bulk forming laxatives and provide primarily the dietary fibre. Dietary fibre consists of unabsorbable plant cell wall and other constituents of vegetable food like cellulose, pectins, glycoproteins and other polysaccharides. Food differs widely in their type and content of fibre, but usual sources of dietary fibre are whole grain, bran, vegetables and fruits.

Mechanism of action

Simple bulk purgatives may act as a laxative by several mechanisms. The simple bulk laxative/purgatives are hydrophilic in nature and are not digested within the GI tract. They absorb water and swell, so an emollient gel is formed. The increased volume or

bulk leads to distension of intestine, with resultant reflex contraction producing peristaltic activity. The faeces remain soft and hydrated. In large intestine, the microfloral fermentation of cellulose and hemicellulose also leads to formation of organic acids and some other products which exert an osmotic effect and thus help/enhance laxative effect. Dietary fibre also supports bacterial growth in colon, which contributes to the faecal mass. Tympanites and a fluid stool often result from the use of simple bulk purgatives.

Bran

Bran is the hard outer layer of grain and is often produced as a by-product of milling in the production of refined grains. Bran obtained from cereal grains including rice, maize, wheat, oats, barley and millet provides water insoluble fibres. Unprocessed wheat bran contains approximately 40% wheat fibre. Bran taken orally provides a laxative/purgative action. Full effect may require daily administration of bran for at least 3-4 days. Unlike a surfactant laxative, bran does not soften the faeces already present in colon or rectum.

Bran is used in simple constipation. It is also used to treat chronic constipation in small animals. Bran is generally safe, but is unpalatable and required usually in large quantity. Flatulence and abdominal distension may occur following bran administration. It should not be used in abdominal pain, vomiting, gut ulcerations, adhesions, stenosis and when faecal impaction is a possibility. Water must be available to the animal all the time when using bran and other bulk laxatives to avoid dehydration and consequent worsening of constipation leading to intestinal obstruction.

Dose

Dose : 1.75 – 7 g (total), PO, daily by addition to food.
Cats : 1.75 g (total), PO, daily by addition to food.

Agar

Agar (Agar-agar) is the dried, hydrophilic, colloidal polysaccharide complex obtained from the agarocytes of algae *Gelidium cartilagineum*. Chemically, agar is a polymer made of two classes of polysaccharide - agaropectin and agarose. Agar is commercially available in the form of a transparent or translucent yellowish-white strip or as fine or granulated powder. It has a slight odour and mucilaginous taste. Agar is used mainly for the preparations of cellulose media and also as an emulsifying agent. Agar is approximately 80% fiber, so it can serve as a bulk purgative when taken orally. As a laxative/purgative, its properties resemble bran.

Ispaghula

Ispaghula (Ispaghul, Isabgol, Psyllium seeds) is the common name used for several members of the plant genus *Plantago* whose seeds are used commercially for the production of mucilage. The unripe and dried seeds of *Plantago ovata*, commonly known as Indian plantago or Isabgol, are widely used as intestinal regulator. The seeds contain a hydrocolloid polysaccharide in the outer seed coat, which is odourless and bland in taste. When soaked in water, the seed coat swells and is surrounded with mucilage. One part of the seeds forms a thick, tasteless jelly with 20 parts of water.

Ispaghula is used in the management of chronic constipation and when excessive rectal straining is to be avoided such as following surgery for perineal hernia repair or anal sac removal. The laxative properties of ispaghula are attributed to the fibre absorbing water and subsequently softening the stool. Due to its ability to increase faecal mass, ispaghula is also used in the control of diarrhoea. It is a useful agent for the treatment of sand impaction in horses. Ispaghula (not bran) is also used in patients those cannot tolerate gluten-containing diets. Its adverse/side effects and contraindications are similar to those of bran.

Dose

Dogs : 3 – 10 g (total), PO, 1 to 2 times daily by addition to moist food.
Cats : 3 g (total), PO, 1 to 2 times daily by addition to moist food.
Horses : Up to 75 g (total), 1 to 2 times daily by addition to moist food.

Sterculia

Sterculia (Karaya gum) is the dried gummy exudates of the tree *Sterculia urens* or *S. tragacanth*, found mainly in India and Africa. The gum exudes naturally or from incisions made to the wood. In water, it is slightly soluble but swells to many times. It has a distinct odour of acetic acid. On oral administration, it takes up water in the GI tract, thereby increasing the volume of faeces and promoting peristalsis. Its action, uses and adverse effects resemble to those of ispaghula.

Dose

Dogs : 1.5 – 6 g (total), PO, 1 to 2 times daily by addition to food or by mouth. Dose may be selected depending on the size of dog.
Cats : 120 mg (total), PO, 2 times daily by addition to food or by mouth.
Kittens : 120 mg, PO, once daily by addition to food.

Methylcellulose and Carboxymethylcellulose sodium

A variety of semi-synthetic derivatives of cellulose including methylcellulose and carboxymethylcellulose sodium are available. These compounds are hydrophilic and indigestible, forming a bulky colloid when mixed with water. When ingested, these agents are not absorbed by the intestines but passes through the digestive tract undisturbed. They attract large amounts of water into the colon, producing a softer and bulkier stool. Presence of inorganic salts increases the viscosity. Their pharmacological properties, side effects and clinical uses resemble to those of other bulk laxatives. In addition, cellulose can bind and reduce the intestinal absorption of many drugs including cardiac glycosides, salicylates and nitrofurantoin. They should be taken with sufficient amounts of fluid to prevent dehydration. Carboxymethylcellulose sodium may contain significant quantity of Na^+ and should not be used when systemic retention of Na^+ and water is a problem. Solutions containing methyl cellulose or similar cellulose derivatives are also used as substitute for tears or saliva if the natural production of these fluids is disturbed.

Dose

Methylcellulose
Dogs : 0.5 – 5 g (total), PO.
Cats : 0.5 – 1 g (total), PO.
Carboxymethylcellulose sodium
Dogs : 3 – 10 g (total), PO.
Cats : 3 g (total), PO.

Polycarbophils

Polycarbophil compounds are synthetic non-absorbable, hydrophilic polyacrylic resins. Polycarbophil calcium is used

mainly as stool stabilizer to treat constipation, diarrhoea and abdominal discomfort. Polycarbophil absorbs about ten times its own weight of water under acidic conditions in stomach, but the swelling ratio markedly increases and reaches 70 times the initial weight at higher pH conditions in intestine. After absorption of sufficient water in intestines, polycarbophil swells to form a soft bulky stool. The bulky mass stimulates the intestinal muscles, speeding stool transit time through the colon. The desirable effect is produced within 12 to 72 hours. Each dose of drug is recommended to be taken with plenty of water (i.e. 1 g with 250 ml of water in human beings).

IV. Osmotic Purgatives

Osmotic purgatives are hypertonic solutions of poorly absorbable substances which retain or attract water from tissues into intestinal lumen mainly by osmotic forces. The resulting distension of intestine by water stimulates mechanoreceptors, which reflexly cause an increase in peristaltic activity. Adequate drinking water must always be provided to the animals. These drugs usually have a latent period of few hours because some time is needed for the osmotic pressure to exert its full effect and to withdraw sufficient water into the lumen. These purgative drugs are contraindicated in weak and dehydrated animals and should not be used in patients with renal failure.

1. Inorganic Salts/Saline Osmotic Purgatives

Some inorganic salts of Mg^{++}, Na^{+} and K^{+} are used as saline osmotic purgatives. All inorganic salts have similar purgative actions; they differ only in dose and risk of systemic toxicity. To produce the desirable purgative effect, all inorganic salts should be given in quantities large enough to create hypertonic concentration in the intestine. Repeated use of saline purgatives may cause fluid and electrolyte imbalance. Saline purgatives are used commonly for preparation of bowel before surgery and colonoscopy, in food/drug poisoning and as after-purge in treatment of tapeworm infestation. Saline purgatives are less reliable in horses and cattle.

Magnesium salts

Magnesium salts (sulphate, hydroxide, carbonate, oxide, peroxide and citrate) are frequently used as saline osmotic purgatives. As the osmotic pressure of saline solutions is related to the number of ions present in the solution, therefore the compounds which are not rapidly absorbed into tissues and yet are freely soluble and ionised exert the strongest and most persistent purgative effect. The best effect, therefore, is exerted by magnesium sulphate (Epsom salt), which is freely soluble, strongly ionised and produces two ions, both of which are only slowly and sparingly absorbed. Magnesium ions also bring about release of cholecystokinin that may aid purgative action of magnesium sulphate.

After oral administration, purgative action of magnesium sulphate is seen after 3-12 hours in monogastric animals and after 12-18 hours in ruminants. Normally in the recommended dose, only 20% of the magnesium ions are absorbed and subsequently eliminated in the urine. If excessive magnesium is absorbed or if renal elimination is impaired, CNS depression and other signs of hypermagnesaemia may become evident. Calcium given intravenously reverses these adverse effects of magnesium. The purgative dose of magnesium salts is more effective when administered on an empty stomach.

In addition to purgative effect, magnesium sulphate has several other uses in medical science. It is available in a gel form for topical application in treating aches and pains. Magnesium sulphate is the first-line antiarrhythmic agent for *torsades de pointes* in cardiac arrest and for managing quinidine-induced arrhythmias. Magnesium sulphate can delay labour in the case of premature labour, to delay pre-mature birth. Magnesium sulphate is also a component of some replacement parenteral fluids and is used mainly to treat hypomagnesaemia. Concentrated solution of magnesium sulphate is occasionally used as euthanizing agent.

Dose

For purgative effect

Magnesium sulphate

Dogs	:	5 – 25 g (total), PO.
Cats	:	2 – 5 g (total), PO.
Pigs	:	25 – 125 g (total), PO.
Horses	:	30 – 100 g (total), PO.
Cattle	:	250 – 500 g (total), PO.

Magnesium hydroxide suspension 8% w/w (Milk of magnesia)

Dogs	:	5 – 10 ml (total), PO.
Cats	:	2 – 6 ml (total), PO.
Horses & cattle	:	1 – 4 L (total), PO.

Sodium salts

Inorganic salts of sodium such as sodium sulphate (Glauber's salt), sodium phosphate, potassium sodium tartrate (Rochelle salt), and even larger quantity of sodium chloride are effective as saline purgative. Their action resembles those of magnesium salts. Sodium saline purgatives should not be used in patients suffering from CHF or other sodium retaining states.

Dose

Sodium sulphate

Dogs	:	0.5 – 2 g (total), PO.
Cats	:	0.3 – 0.6 g (total), PO.
Pigs	:	15 – 30 g (total), PO.
Sheep	:	10 – 15 g (total), PO.
Cattle	:	60 – 120 g (total), PO.
Horses	:	30 – 60 g (total), PO.

2. Carbohydrate osmotic purgatives

Lactulose

Lactulose is a disaccharide, a synthetic derivative of lactose, containing one molecule of galactose and one molecule of fructose. It occurs as a white powder that is very less soluble in alcohol and very soluble in water. The commercial syrup used for treatment of constipation is often dyed yellow-orange.

Mechanism of action and pharmacological effects

On oral administration, lactulose is not digested by mammalian gut enzymes, but is metabolised in colon by saccharolytic

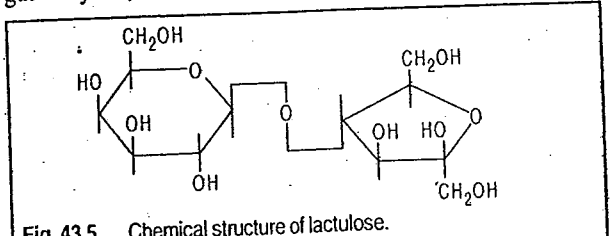


Fig. 43.5. Chemical structure of lactulose.

microflora to produce acetic, formic, lactic and other organic acids. These organic acids lower the pH of the colonic content and exert an osmotic effect. The concomitant reduction in luminal pH also may enhance motility and secretion. The increased osmotic activity results in modest accumulation of fluid and passage of soft-formed faeces in 1-3 days. The added volume of gas and flatulence caused by fermentation of the lactulose adds to the volume of the faecal material and makes it easier to expel. The acidification of colon by lactulose also causes ammonia (NH_3) to migrate from the blood into the colon, where it is trapped as NH_4^+ (ammonium ion) and expelled with the faeces. So lactulose is also used to reduce blood NH_3 in patients with portal hypertension and hepatic encephalopathy associated with chronic liver disease.

Pharmacokinetics

The pharmacokinetic data on lactulose in domestic animals are scarce. In humans, less than 3% of an oral dose of lactulose is absorbed from the small intestine and excreted unchanged in the urine within 24 hours.

Side effects/Adverse effects

Use of lactulose may result in flatulence, cramps and abdominal discomfort, especially when therapy is initiated. Nausea and vomiting also have been reported, particularly with high dosages. Excessive dosage can cause diarrhoea, loss of fluid and potassium and hypernatraemia. Cats dislike the taste of lactulose so its administration may be difficult in this species. In common with other osmotic laxatives, lactulose can cause electrolytic imbalances in sensitive animals.

Drug interactions

Non-systemic oral antacids may reduce the colonic acidification effects on lactulose. Orally administered antibiotics could eliminate the bacteria responsible for metabolising lactulose, thereby reducing it efficacy.

Clinical uses

The primary use of lactulose is to reduce blood ammonia levels in the prevention and treatment of hepatic encephalopathy in small animals. It is also used as an osmotic purgative in constipation in small animals. It is used to maintain soft faeces in animals (especially cats) those are prone to constipation.

Dose

Lactulose oral solution (0.62 – 0.74 g/ml)

Dogs & cats : 0.25 – 0.5 ml/kg, PO, 2 to 3 times daily.

Lactitol

Lactitol is a sugar alcohol commonly used as a replacement bulk sweetener for low calorie foods. Lactitol is used as an alternative osmotic purgative. It is less sweet than lactulose and may be better tolerated. Lactitol is regarded as a safe agent, like most other sugar alcohols (with the exception of erythritol), but can cause cramping, flatulence and diarrhoea in some individuals.

Glycerine

Glycerine (Glycerin, glycerol) is a trihydric alcohol [$C_3H_5(OH)_3$] obtained by hydrolysis of fats and fixed oils. It is clear colourless hydroscopic syrupy liquid that is miscible with water and alcohol. When administered rectally (as a suppository), it acts mainly by its osmotic effect to soften and lubricate the passage of inspissated faeces. It may also stimulate target intestinal contractions by local irritation to produce desirable effect. Glycerine promotes colonic evacuation in about 30 minutes.

Dose

Dogs & cats : 1 – 2 ml/kg (of 50% sol.), PO. Dose may be repeated once at 8 hours.

Sorbitol and Mannitol

Sugar alcohols such as mannitol and sorbitol are occasionally used as osmotic purgatives. When given orally, they are poorly absorbed and fermented in the terminal ileum and large intestine. When sodium polystyrene sulphonate is utilised in the therapy of hyperkalaemia, sorbitol is frequently included to combat the constipating action of cation-exchange resin. Sorbitol as purgative is often mixed with activated charcoal in the management of poisoning or drug overdoses.

Dose

Sorbitol (70% solution)

Dogs & cats : 3 ml/kg, PO, 2 times daily.

Macrogol

Macrogol (Polyethylene glycol, PEG) is a polyether compound. Macrogols have been used as laxatives i.e. to treat constipation. Various forms of macrogol viz. Macrogols 4000, 3350 and 600 are available; the number represents the average molecular weight of the polyethylene glycol. Macrogols have been used as laxatives to treat constipation. They are also used as excipients in pharmaceutical products.

V. Irritant/Stimulant Purgatives

Irritant (Stimulant purgatives, contact purgatives) are powerful purgatives which produce action mainly through irritation of GI tract. These purgatives are regarded as being direct-acting or indirect acting depending on whether a metabolic alteration is first required to form an active product. Some agents are highly irritant and may cause severe colic and superpurgation; so they have been grouped into a separate class of drastic purgatives. Large doses of stimulant purgatives can cause purgation with excessive fluid and electrolyte imbalance. Regular use of stimulant purgatives causes hypokalaemia. The routine and long-term use of irritant purgative is discouraged because it may lead to colonic atony.

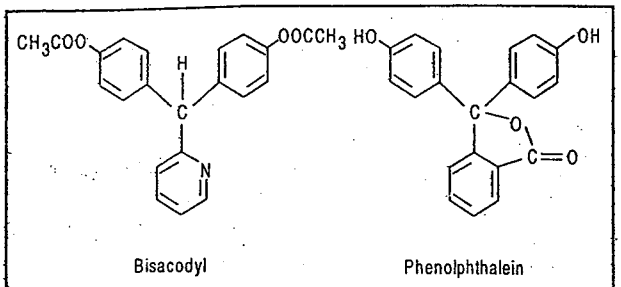
Mechanism of action

Irritant/stimulant purgatives were initially thought to act by stimulating the intestinal mucosa and motor activity by causing irritation. Though some of these agents do primarily increase intestinal motility by stimulating intestinal mucosa and myenteric plexus, the more important mechanism of action is now thought to be accumulation of water and electrolytes in the lumen by alteration of absorptive and secretory activity of the mucosal cells. They inhibit Na^+-K^+-ATPase at the basolateral membrane of villous cells, which could account for at least a portion of their laxative effect. They reduce net absorption of electrolytes and water and increase the permeability of mucosa, possibly by making tight junctions leaky. Some of the agents have been reported to increase the synthesis of prostaglandins and cAMP, which also contribute to increased secretion of water and electrolytes.

1. Direct Irritant Purgatives

a. *Diphenylmethanes*

Diphenylmethanes is a group of organic compounds with the formula $(C_6H_5)_2CH_2$. These compounds possess a common structure consisting of a methane group wherein two hydrogen atoms are replaced by two phenyl groups. Some o



Bisacodyl Phenolphthalein

Fig. 43.6. Chemical structures of selected direct irritant purgatives.

diphenylmethane agents, including bisacodyl and phenolphthalein are widely used as purgatives.

Bisacodyl

Bisacodyl is a diphenylmethane laxative that occurs as a white to off-white crystalline powder. It is practically insoluble in water and sparingly soluble in alcohol. Bisacodyl as direct irritant purgative is more popular than phenolphthalein.

Mechanism of action

Like other irritant purgatives, the exact mechanism of action of bisacodyl is not clear. It is thought to produce purgation by inhibiting glucose and Na^+-K^+-ATPase activity as well as direct stimulation of the colonic smooth muscles and myenteric plexus. It is also a contact laxative; it increases fluid and NaCl secretion.

Pharmacological effects and Pharmacokinetics

Bisacodyl is administered both orally and per-rectally. It is minimally absorbed after oral or rectal administration. Onset of action after oral administration is generally seen after 6-10 hours and in 15-60 minutes after rectal administration. Bisacodyl is rapidly converted by intestinal and bacterial enzymes to its active desacetyl metabolite. As much as 5% of an orally or per rectally administered dose is absorbed and excreted in the urine as the glucuronide conjugate.

Side effects/Adverse effects

Bisacodyl shows relatively few side effects after therapeutic doses like occasional cramping, nausea and/or diarrhoea. Overdosage may result in severe cramping, diarrhoea, vomiting and potential fluid and electrolyte imbalance. The diphenylmethanes (phenolphthalein and bisacodyl) can damage enterocytes and can initiate an inflammatory response in the colon. Moreover, allergic reactions, osteomalacia and protein losing gastroenteropathy have been reported following excessive use of diphenylmethanes. Long-term use with bisacodyl can result in damage to myenteric plexus.

Contraindications and precautions

Bisacodyl and other irritant purgatives should not be used in presence of severe constipation (impaction) or obstructive lesions. Stimulant laxatives may potentially decrease GI transit time thereby affecting absorption of other oral drugs.

Clinical uses

Bisacodyl is clinically used to treat constipation. It is a useful adjunct to enemas for the treatment of mild to moderate constipation. It is also used to stimulate defecation prior to colonoscopy, but is rarely used in chronically constipated dogs and cats.

Dose

Dogs : 5 – 20 mg (total), PO, once daily. Dose should be selected on the basis of body weight.

Cats : 5 mg (total), PO, once daily.

Dogs & cats : 1 – 2 ml of enema (bisacodyl enema 10 mg/3 ml)

: 1 – 3 suppositories (5 mg rectal suppository).

Phenolphthalein

Phenolphthalein is a chemical compound with the formula $C_{20}H_{14}O_4$. It exists as white powder and is used primarily as a laboratory indicator. It is used also as a purgative and has good action with more pronounced effect in primates and swine. Its exact mechanism of action is not clear, but it possesses large intestine irritant properties. After oral administration, phenolphthalein acts on large intestine and produces purgative effects after 6-8 hours. A portion (about 15%) of the drug undergoes entero-hepatic cycle for few days, which can produce a longer action. So this compound is useful also in chronic constipation.

Phenolphthalein is used mainly for constipation and chronic constipation. In normal doses, it does not produce much serious side effects. Excessive purgation with loss of fluid and electrolyte may occur with overdosage. Faeces and urine may show pink or reddish tinge, if reaction are alkaline.

Dose

Dogs & cats : 10 mg/kg, PO.

Pigs : 1 – 2 g (total), PO.

b. Inorganic Compounds

Several inorganic compounds like metallic mercury, mercurous chloride (calomel) and sulphur were once used as direct irritant purgative. However, the use of inorganic compounds as purgatives in veterinary medicine has very much declined mainly due to their toxic effects and availability of better compounds.

2. Indirect Irritant Purgatives

Unlike direct irritant purgatives, the indirect irritant purgatives require bioactivation to exert an irritant and purgative action.

a. Vegetable Oils

Several bland vegetable oils such as castror oil and linseed oil act as irritant purgatives. Their action is based on hydrolysis by pancreatic lipase in the small intestine and subsequent formation of sodium and potassium salts of the released fatty acids. Thus they form sodium and potassium soaps which are soluble and irritant in nature. This saponification process also produces glycerol as a by-product, which acts as a lubricating and osmotic purgative thus assisting in breaking up of faecal masses and in stimulating defecation. Efficacy and potency of active product formed depends on the type of oil used.

Castor oil

Castor oil is a vegetable oil obtained from the seeds of castor plant *Rinicus communis*. It is a triglyceride in which approximately 90 percent of fatty acid chains are ricinoleic acid. Castor oil is one of the oldest known purgatives. Castor oil exists as a colourless to very pale yellow liquid with mild or no odour or taste.

Mechanism of action

On oral administration, the castor oil triglyceride is hydrolysed in the ileum by pancreatic lipase to ricinoleic acid (ricinoleate) and glycerol. Ricinoleate is believed to irritate the mucosa and stimulate intestinal contractions. In addition to this, castor oil is believed to decrease intestinal absorption of water and electrolytes and enhance secretion by a detergent like action on the mucosa (action produced like anionic detergents). The lubricant action of castor oil may also contribute to softening of faeces and in facilitating purgation.

Pharmacological effects

Castor oil releases ricinoleate in the small intestine that acts rapidly and relatively completely causing accumulation of fluid and evacuation of faeces. The purgative effect occurs in 4-8 hours in small animals and 12-18 hours in large animals. Colonic emptying is so complete that normal functioning of bowel movement may require several days. Ricinoleic acid being polar is poorly absorbed.

Side effects/Adverse effects

Castor oil is a potent irritant purgative that produces abdominal cramps, colic and dehydration with electrolyte imbalance. Structural damage to villous tips with other gross morphological damage to the intestinal epithelium has been reported. Castor oil should not be used in pregnant animals as its strong action also produces uterine contractions. For these reasons and because of possible reduction of the absorption of nutrients, long-term use of castor oil must be avoided.

Clinical uses

Castor oil is used as purgative mainly in non-ruminants and often in calves (before the rumen is developed) and foals. Moist bulky feeds are needed following purgation with castor oil. It should not be used in chronic constipation, where it can aggravate the condition after a temporary relief.

Dose

Dogs	:	5 – 25 ml (total), PO.
Calves & foals	:	30 – 90 ml (total), PO.
Pigs	:	30 – 180 ml (total), PO.
Horses	:	250 – 1000 ml (total), PO.

Linseed oil

Linseed (flax seed) oil is a vegetable oil obtained from the dried ripe seeds of the flax plant (*Linum usitatissimum*, Linaceae). Similar to castor oil, it acts by releasing a fatty acid release of linolenic acid (linoleate) that acts indirectly to produce purgation. However, linoleate formed is less irritant and less potent than the ricinoleate formed by castor oil. Therefore purgation produce by linseed oil is considered to be of moderate intensity. Unlike castor oil, linseed oil can be used as purgative in adult ruminants.

Dose

Dogs	:	8 – 15 ml (total), PO.
Sheep & goats	:	120 – 180 ml (total), PO.
Horses	:	500 – 750 ml (total), PO.
Cattle	:	500 – 1000 ml (total), PO.

b. Anthraquinone/ Anthracene Purgatives

Anthraquinone, also called anthracenedione or dioxoanthracene, is an aromatic organic compound with formula $C_{14}H_8O_2$. Anthraquinone is viewed as a 9,10 diketone derivative of anthracene, a polycyclic aromatic hydrocarbon consisting of three fused benzene rings. Anthraquinone is a building block of many dyes and is also present in many plants. Anthraquinone or its derivatives (together called anthraquinones, anthracenes or emodines) when taken orally produces purgative action by acting indirectly in large intestine. The clinically used anthraquinone purgatives are mainly of the plant origin, but a few synthetic compounds are also available.

i. Vegetable Anthraquinone Purgatives

Vegetable anthraquinone purgatives contain precursor glycosides. After oral administration, a part of these glycosides remain unabsorbed in intestine and is metabolised by the bacterial enzymes in large intestine causing release of the active aglycone called anthraquinones or emodines, which are di- tri-, or tetra–hydroxyanthraquinone or their modifications. The action of active anthraquinones is mainly seen on the large intestine, where myenteric plexus is stimulated. The main members of this group are aloes, cascara sagrada, senna and rhubarb.

Aloes (Aloe)

Aloe is a genus containing about four hundred species of flowering succulent plants, frequently cultivated as ornamental plants both in gardens and in pots. Some species of genus *Aloe*, particularly *A. vera (A. barbadensis) and A. ferox*, possess significant medicinal properties and are used both internally and externally in human and animals. The dried juice of the leaves of plant *Aloe* is available as opaque masses, having colour ranging from reddish black to brownish black. Aloes (term used for any of various plants of the genus *Aloe*) was the most widely used anthracene purgative and the prototype drug of vegetable anthraquinones until the introduction of synthetic anthraquinones.

Mechanism of action and Pharmacological effects

Aloes contains anthraquinone glycosides, which after oral administration mainly remains unabsorbed in GI tract, but get metabolised in large intestine to release the active anthraquinones such as aloe-emodin and aloin. Some anthraquinone glycosides are also absorbed from the intestine and then re-excreted into the large intestine, where they produce irritation and increased peristalsis. Because of this complex process, purgation is delayed for about 12-18 hours (also depends on the presence of food in the GI tract) and effect lasts for up to 24 hours. The anthaquionones are largely excreted in faeces. Some absorbed anthraquinones are also excreted in urine, saliva, bile and even in milk.

Side effects/Adverse effects

The anthraquinone purgatives including aloes may produce an excessive purgative effect and abdominal pain. Large doses are reported to produce nephritis. Aloes is contraindicated in hyperpyrexia, gastro-enteritis, peritonitis, inflammatory conditions of genitourinary tract, pregnancy and lactation.

Clinical uses

Aloes is used as a purgative, especially in animals those suffer from impaction of the large intestine (as active metabolites are excreted in large intestine). The horses with their numerous colon flexures may benefit more from such purgatives. All non-ruminants also respond well to anthraquinone purgatives. Extract of *Aloe vera* also possesses some antibacterial and antifungal activities and is used topically in treatment of wounds.

Dose

Dogs	:	0.6 – 2 g (total), PO.
Horses	:	15 – 30 g (total), PO.
Cattle	:	30 – 60 g (total), PO.

Cascara sagrada

Cascara sagrada is the common name of the dried, aged bark of a species of buckthorn plant, with the botanical name *Rhamnus purshiana*. It has a characteristic odour with a nauseating bitter taste. Cascara contains many glycosides (cascarosides A, B, C & D), which release the active anthraquinone aglycones (e.g. emodin, oxanthrone and frangulaemodin), after metabolism into the large intestine. The pharmacological properties and action of cascara resemble those of aloes.

Dose

Dry extract of cascara sagrada

Dogs : 0.12 – 0.15 g (total), PO.

Liquid extract of cascara sagrada

Dogs : 2 – 4 ml (total), PO.

Senna

Senna is a large genus of flowering plants in the family Fabaceae. Some species of *Senna* (together called sennas) are medicinally used. Senna acts as purgatives and is similar to aloes in having as active ingredients anthraquinone derivatives and their glycosides, called sennosides. Senna prepared from the dried leaflets of *Senna alexandrina (Cassia senna, Cassia acutifolia)* is used in modern medicine as a laxative. It contains several anthraquinone glycosides, which release active anthraquinones like sennidin A and B, rhein and aloe-emodin. Senna acts on lower bowel and increases the peristaltic movements of the colon by irritating the colonic mucosa. The type of action produced i.e. laxative or purgative, depends on the dose. Senna imparts a yellow to red colour to the urine.

Dose

Senna powder

Dogs : 2 – 8 g (total), PO.

Horses : Up to 125 g (total), PO.

Rhubarb

Rhubarb (Rheum) is a group of plants which belong to the genus *Rheum* in the family Polygonaceae. The leaves of the rhubarb are toxic, but other parts of the plants have culinary and medicinal uses. The dried rhizome and roots of *Rheum officinale* and some other species of *Rheum* are used for purgative action. Rhubarb contains free anthraquinones and their glycosides. Important anthraquinones are chrysophanic acid, rhein and aloe-emodin. It also contains a high proportion of tannic acid, which exerts a constipating effect following the initial purgative effect of the anthraquinones. Rhubarb may stain alkaline faeces and urine reddish brown.

ii. Synthetic Anthraquinone Purgatives

Dantron

Dantron (Danthron, Danthrone, 1, 8-Dihydroxyanthraquinone) is a synthetic anthraquinone purgative, which has been used in veterinary medicine. Although 1,8-dihydroxyanthraquinone is a natural constituent of certain drugs; it is difficult to isolate it in a pure state. Unlike vegetable products, dantron has advantage of having standard unvarying composition and absorption rate in a given condition. Its smaller doses are laxative and larger doses are purgative. The effect of dantron is evident within 6-14 hours in small animals and 12-36 hours in large animals.

Prolong administration of dantron may cause degeneration of the myenteric plexus leading to loss of intestinal motility. Repeat dosing should be avoided in large animals, especially in horses because of long latent period and possibility of severe superpurgation. Sufficient anthraquinone is excreted in milk that may affect nursing young ones. Although clinical evaluation of long-term toxicity of the anthraquinones is conflicting, recent studies have associated dantron with appearance of hepatic and intestinal tumours in laboratory animals. The naturally occurring glycosides seem to differ in this regard. The pharmacological actions, other adverse effects, and clinical uses of dantron are similar to those of aloes.

Dose

Dogs & cats : 0.15 – 3 g (total), PO.

Foals & calves : 3 – 5 g (total), PO.

Horses and cattle : 10 – 30 g (total), PO.

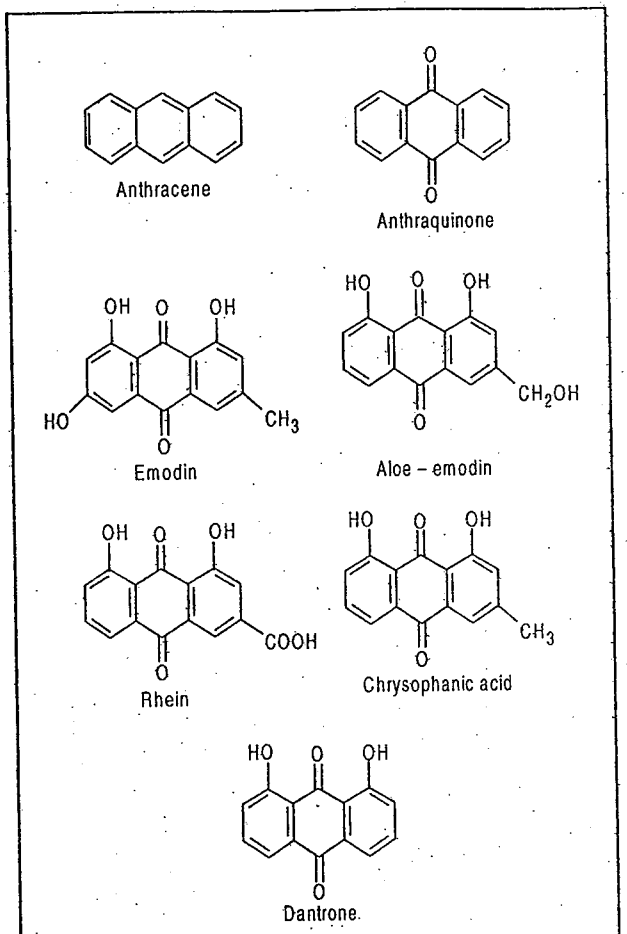


Fig. 43.7. Chemical structures of selected indirect natural and synthetic anthracene purgatives.

3. Drastic Irritant Purgatives

Drastic irritant purgatives are highly irritant compounds which may cause severe colic and superpurgation. Known as drastic, they are dangerous compounds and no longer used in therapy. Some important compounds and their sources have been given in Table 43.1

Table 43.1: List of selected drastic purgatives and their source.

Compound	Source
Jalap	Dried tubercles or roots of *Ipomoea purga.*
Colocynth	Dried fruit pulp of *Citrullus colocynthis.*
Podophyllum	Dried rhizome and root of *Podophyllum peltatum* Linn.
Croton oil	A fixed oil obtained from seeds of *Croton tiglium.*
Barium chloride	Chloride salt of barium.

VI. Neuromuscular Purgatives

Neuromuscular purgatives are cholinergic agents with muscarinic receptor agonistic activity. They initiate hypermotility and promote secretion of the GI tract. Neostigmine, physostigmine, bethanechol and carbachol have been used for this purpose. Peristaltic activity increases within 10 – 30 minutes following their parenteral administration. These agents should not be used or used carefully when a mechanical obstruction is present or viability of bowel is doubtful. They may also precipitate existing colic. Overall, these drugs are not commonly used nowadays because of their excessive intestinal contraction and severe systemic effects on the cardiovascular system. The detailed actions and pharmacology of these agents have been given in Chapter 13.

Carbachol

Carbachol is a synthetic choline ester with parasympathomimetic actions. Carbachol has been used to assist peristalsis in horses, but it also produces sweating and salivation which are not desirable. In cattle, some stimulant effect is exerted on the rumen and is used to recommence rumination when stasis has occurred (see also Chapter 13).

Dose

Horse & cattle : 2 – 5 mg (total), SC.

Foals : 0.5 – 1 mg (total), SC.

VII. Peripheral Opioid Antagonists

Alvimopan

Alvimopan is a peripherally acting μ-opioid receptor antagonist. Alvimopan competitively binds to mu-opioid receptor in the gastrointestinal tract and minimises the constipating effect of opioid analgesics. It has limited ability to cross the blood-brain barrier, so many of the undesirable side effects on CNS are not observed. It is mainly recommended for use in human patients to avoid postoperative ileus following partial GI tract surgery. It helps the bowel recover more quickly by protecting the intestine from the effects of opioid analgesics which are used to treat pain after surgery.

Methylnaltrexone

Methylnaltrexone (MNTX) is a recently introduced peripherally-acting μ-opioid receptor antagonist. Similar to alvimopan, methylnaltrexone has primarily peripheral action on GI tract. It is used for the treatment of opioid induced constipation and is recommended only when ordinary laxatives have failed.

VIII. Prostanoids

Lubiprostone

Lubiprostone is a prostaglandin E_1 derivative, with preferential activity on GI tract. It acts by specifically activating ClC-2 (Chloride channel protein 2) chloride channels on the apical aspect of gastrointestinal epithelial cells, producing a chloride-rich fluid secretion. An efflux of chloride ions into the lumen, in turn leads to an efflux of sodium ions through a paracellular pathway to maintain isoelectric neutrality. As a result, water follows sodium into the lumen in order to maintain isotonic equilibrium, thereby increasing intestinal fluid secretion. These secretions soften stool, increase GI motility and promote spontaneous bowel movements. Lubiprostone is primarily recommended in the management of idiopathic chronic constipation, which is generally characterised by infrequent or difficult passage of stool. Unlike many laxatives, lubiprostone does not produce tolerance, dependency or altered serum electrolyte concentration.

IX. Enema/Enemata

Introduction of solutions or suppositories into the rectum to initiate defecation reflex is a useful and simple method to correct or prevent constipation. Many preparations have therapeutic importance as enemas including soapy water (soft anionic soap), isotonic or hypertonic sodium chloride solutions, sodium citrate, sorbitol, glycerine, surfactants (e.g. sodium lauryl sulphoacetate), mineral oils and vegetable oils (e.g. olive oil). Enema preparations containing phosphate (sodium acid phosphate and sodium phosphate) are also useful, but these should not be used indiscriminately as they can precipitate potentially fatal hyperphosphataemia, hypocalcaemia and hypernatraemia in cats and debilitated animals. For the removal of impacted rectal and colonic contents in dog or in treatment of colic, it is preferable to infuse large volumes of warm fluid per rectum than to rely solely on a purgative. The lubrication, distension and, in some cases, mild irritation produced by the fluid softens the faecal mass and stimulates peristalsis. Warm, soapy water or glycerine is frequently used for this purpose. The dosage of commonly used substances for inducing enema in small animals is given in Table 43.2.

ANTIDIARRHOEAL DRUGS

A number of drugs are used in the treatment and management of diarrhoea. The treatment of diarrhoea is generally non-specific and usually aimed at reducing the discomfort and inconvenience

Table 43.2: Dosage of selected compounds used for enemas in small animals.

Compound	Dogs	Cats
Enema fluid (soapy water, warm water, warm isotonic saline)	5 – 10 ml/kg.	5 – 10 ml/kg.
Liquid paraffin	0.25 – 0.5 ml/kg.	0.25 – 0.5 ml/kg.
Sterile lubricant jelly	10 – 20 ml mixed with warm water.	5 – 10 ml mixed with warm water.
Docusate liquid		
Docusate sodium	5 – 30 ml (depending on body weight).	5 – 10 ml alone or added to warm water.
Docusate calcium	10 – 30 ml alone or added to warm water.	5 – 10 ml alone or added to warm water.
Bisacodyl	0.25 mg/kg.	0.25 mg/kg.
Lactulose solution	0.25 – 0.5 ml/kg.	0.25 – 0.5 ml/kg

of frequent bowel movements. Non-specific drugs help also to reduce excessive fluid and electrolyte loss. Specific chemotherapeutic agents are required when diarrhoea is induced by pathogens.

Diarrhoea

Diarrhoea is defined as an abnormal increase in the frequency, fluidity, or volume of faeces resulting from excessive faecal water content. Diarrhoea may result from variety of pathophysiological processes including an increased number of osmotically active particles in intestinal lumen, stimulation of intestinal fluid and ion secretion, breakdown in intestinal mucosal permeability, and alterations in intestinal motility. Any insult to the gut that disrupts normal intestinal physiology can result in diarrhoea either due to increased secretion or decreased absorption of fluid and ions by the intestine. The causative agents of diarrhoea are numerous and may include dietary imbalance, hypersensitivity, poisoning, infections, toxins, neoplasia, villous atrophy, inflammation, exocrine pancreatic insufficiency and stress. Diarrhoea may also result as a side effect of a number of therapeutic agents. Treatment of diarrhoea should be directed at the primary cause; however due to multifactorial aetiology of the condition, the causative agent(s) cannot always be ascertained, especially in the acute diarrhoea. Therefore, symptomatic treatment and management of diarrhoea is important and include bowel rest and correction of fluid, electrolyte and acid-base disturbance.

Classification

There are numerous antidiarrhoeal drugs used in veterinary medicine. These include non-specific antidiarrhoeal drugs and specific chemotherapeutic drugs.

I. Non-specific antidiarrhoeal drugs
1. Gastrointestinal protectants and adsorbents
   e.g. kaolin, pectin, bismuth salts, activated charcoal and colestyramine.
2. Antimotility drugs
a. Opioids
   e.g. tincture of opium, codeine, diphenoxylate and loperamide.
3. Antisecretory and anti-inflammatory drugs
a. Salicylic acid derivatives
   e.g. sulphasalazine, mesalazine, olsalazine and balsalzide.
b. Anticholinergic drugs
   e.g. atropine, hyoscine, aminopentamide, glycopyrronium and oxyphenonium.
c. Miscellaneous antisecretory drugs
   e.g. NSAIDs, glucocorticoids, clonidine, octreotide and racecadotril.
4. Miscellaneous drugs
   e.g. tannic acid, catechu, carboxymethylcellulose and psyllium.

II. Specific chemotherapeutic agents
   e.g. streptomycin, neomycin, ampicillin, succinyl-sulphathiazole, enrofloxacin, nitrofurazone and furazolidone.

I. NON-SPECIFIC ANTIDIARRHOEAL DRUGS

1. Gastrointestinal Protectants and Adsorbents

Gastrointestinal protectants (Protectives) and adsorbents are substances which either coat the mucosal surface or adsorb the toxic compounds in GI tract. The protectants produce a coating on the GI epithelium and prevent irritation and erosion of the mucosa. Adsorbents given by mouth adsorb toxins from the GI tract and prevent their absorption which are subsequently eliminated in the faeces. Many compounds possess both properties to varying degree and are used in the treatment of diarrhoea. Most of these agents have little or no effect on fluid or electrolyte losses and their value in diarrhoea is doubtful.

Kaolin

Kaolin, also called China clay, is a naturally occurring hydrated aluminium silicate, which is powdered and refined for pharmaceutical use. Kaolin is a white, odourless and almost tasteless powder that is practically insoluble in water. It has been used for centuries as a symptomatic treatment of diarrhoea. After its oral administration, kaolin remains unabsorbed in the intestine. It is thought to possess adsorbent and protective qualities; however, its efficacy is in question. It is reported that kaolin does not decrease faecal water in diarrhoea and may actually enhance the intestinal secretion of sodium. It may bind also other antidiarrhoeal drugs and reduce their efficacy.

Side effects/Adverse effects

At usual doses, kaolin has no adverse effects. Constipation may occur in high doses that may cause faecal impaction in old and debilitated animals.

Clinical uses

Although its efficacy is in question, kaolin is used in veterinary medicine primarily as an oral anti-diarrhoeal agent. It has also been used as an adsorbent and GI protective agent following ingestion of certain toxins. Kaolin is often given in combination with pectin.

Dose

Dogs	:	Up to 8 g (total), PO.
Calves & foals	:	15 – 60 g (total), PO.
Cattle & horses	:	50 – 200 g (total), PO.

Pectin

Pectin is a carbohydrate polymer consisting primarily of partially methoxylated polygalacturonic acids and is obtained from the inner rind of citrus fruits or from apple pomace. It is a course or fine, yellowish-white, almost odourless compound. One gram of pectin is soluble in about 20 ml of water and forms a viscous colloidal solution. Like kaolin, pectin possesses both adsorbent and protective properties. In addition, pectin components by forming galacturic acid have been demonstrated to decrease pH in the intestinal lumen. After oral administration, pectin remains unabsorbed in GI tract and is decomposed (about 90%) in the intestine.

Similar to kaolin, the efficacy of pectin in diarrhoea is doubtful. In veterinary practice, pectin and kaolin are used together in an oral suspension formulation for treatment of diarrhoea. Pectin is also used occasionally in preparations of internal emulsions. It is also employed for preparing or stabilising pharmaceutical and cosmetic lotions and creams. At usual doses, pectin generally has no adverse effects. Overdosage is also unlikely to cause any serious effects, but constipation requiring treatment may occur.

Dose

Kaolin and pectin suspension (Light kaolin 200 mg and pectin 4.3 mg per ml).

Dogs & cats	:	1 – 2 ml/kg, PO, 2 to 3 times daily.
Horses	:	1 ml/kg, PO, 2 to 3 times daily.

Calves : 1 – 2 ml/kg, PO, 2 to 3 times daily.
Birds : 2 ml/kg, PO, 2 to 4 times daily.

Bismuth subsalicylate

Several bismuth salts including subsalicylate (salicylate), carbonate, subnitrate and subgallate are used in diarrhoea, and occasionally for nausea and vomiting. Among these, bismuth subsalicylate has substantial antidiarrhoeal activity, especially in animals with acute diarrhoea. Bismuth subsalicylate is a crystalline 1: 1 ratio of trivalent bismuth and salicylate compound.

Pharmacological effects

Bismuth subsalicylate has been shown to possess both antisecretory and antibacterial activity in several species. After oral administration, it is chemically transformed throughout the GI tract to bismuth and salicylate. The salicylate/subsalicylate portion possesses antisecretory and anti-inflammatory effect due to its anti-prostaglandin action (blocks prostaglandin synthetase), which helps in controlling diarrhoeal syndrome. The bismuth portion possesses antibacterial activity against several pathogens and also adsorbs bacterial enterotoxins. In humans and cats, nearly all the salicylate is systemically available. The bismuth is known to accumulate in the kidney.

Side effects/Adverse effects

At normal doses, bismuth subsalicylate lacks serious side effects. However, oral administration results in black stools that resemble melena. Most dogs and cats dislike the taste of drug and do not willingly take it. The drug should probably not be given to cats due to the systemic absorption and toxicity of salicylates. More importantly, the salicylate portion may cause nephrotoxicity, especially if the other potentially nephrotoxic drugs (e.g. aminoglycosides) are used simultaneously. Bismuth subsalicylate should not be used in animals with bleeding tendencies.

Clinical uses

Bismuth subsalicylate is useful in the treatment of mild to moderate diarrhoea. It is also employed as a gastric and intestinal sedative and antiseptic. It adsorbs also *E. coli* enterotoxins and has been shown to be effective (in high doses) in preventing enterotoxigenic 'traveller's diarrhoea' in man.

Dose

Bismuth subsalicylate powder

Dogs : 0.3 – 1 g (total), PO.
Cats : 0.1 – 0.3 g (total), PO.
Foals & calves : 0.8 – 4 g (total), PO.
Cattle & horses: 15 – 30 g (total), PO.

Bismuth subsalicylate suspension (Bismuth subsalicylate 17.5 mg/ml)

Dogs & cats : 1 – 3 ml (total), PO, daily in divided doses.
Foals & calves : 0.5 ml/kg, PO, 2 times daily.
Horses : 1 – 4 ml/kg, PO, 2 times daily.

Activated charcoal

Activated charcoal occurs as a fine, black, odourless and tasteless powder that is insoluble in water or alcohol. It is prepared by burning certain wood in the absence of air. Charcoal preparations vary according to the source of base material, surface area, capacity for drug binding, and affinity and avidity of drug binding. Source materials are usually lignite, wood or peat. Activation forms more pores and enlarges surface area and makes the charcoal more efficacious. Activation time is directly correlated to the molecular size of the compounds adsorbed. Because most drugs are of an intermediate molecular weight, charcoal with pore sizes between 10 and 20 Da are most appropriate.

Pharmacological effects

Activated charcoal acts as an excellent adsorbent and intestinal astringent. It adsorbs many chemicals and drugs in the upper GI tract and prevents or reduces their absorption. It forms a stable complex with these substances, which permits their evacuation from the body. Charcoal does not show any specific action on tissues. Activated charcoal is neither absorbed nor metabolised in the body.

Side effects/Adverse effects

Activated charcoal is very safe as toxic effects have not been reported even in overdosages. However, very rapid administration of charcoal may induce vomiting. Charcoal powder is highly staining and colours faeces black. Charcoal should not be administered with other therapeutic agents, dairy products or mineral oil as it adsorbs these agents and in turn its own efficacy may also decrease.

Clinical uses

Activated charcoal is administered orally to adsorb certain drugs or toxins to prevent their systemic absorption. Because of its adsorptive activity and rapidity of action, activated charcoal is valuable for treatment of certain poisonings (see also Chapter 8) in many species. However, its clinical efficacy as antidiarrhoeal agent is limited.

Dose

Dogs & cats : 10 ml/kg of slurry (1g/5 ml of water), PO.
Ruminants : 1 – 3 g/kg, PO (1 g of charcoal in 3-5 ml of water). Charcoal is usually administered via stomach tube.

Colestyramine

Colestyramine (Cholestyramine) is a bile acid sequestrant, which binds bile in the gastrointestinal tract and prevents its reabsorption. It is a strong basic anion exchange resin, which helps to exchange its chloride anions with anionic bile acids in the gastrointestinal tract and binds them strongly in the resin matrix. In order to increase the number of binding sites, colestyramine is attached to a polystyrene matrix, which acts as a non-specific adsorbent. Although primarily used to treat hypercholesterolemia and pruritus associated with increased bile acids, colestyramine has also been used to symptomatically treat diarrhoea. Nausea, constipation, steatorrhoea and decreased fat-soluble vitamins absorption are reported undesirable effects. Colestyramine should be administered in food or water.

Dose

Dogs & cats : 200 – 300 mg/kg, PO, 2 times daily.

Other agents

Many other drugs possess protective and adsorbent properties to varying degree and are considered useful in diarrhoea. These include magnesium trisilicate, hydrated magnesium aluminium trisilicate (activated attapulgite), aluminium hydroxide and phosphate, calcium carbonate and zinc oxide. Many antidiarrhoeal preparations are available in market, which have combinations of two or more adsorbents and protectants. These include combinations of kaolin and pectin, bismuth carbonate and calcium carbonate, attapulgite and bone charcoal, and bismuth subnitrate, calcium phosphate, charcoal and kaolin.

2. Antimotility Drugs

Hypermotility is known to contribute in several diarrhoeal syndromes in which shortened intestinal transit time is associated with decreased resorption of electrolyte and water. Intestinal transit time is determined by the ratio between peristalsis and segmentation contractions. Peristaltic activity mediated by longitudinal and circular muscles is involved mainly in propulsive movements (also helps in mixing and absorption), whereas rhythmic segmentation due to simple non-propulsive contractions of circular muscles provides resistance to and slows aboral movement of ingesta and thus promotes mixing and digestion of food. In hypomotility diarrhoea (in some forms of diarrhoea, intestinal motility is decreased rather than increased) due to diminished rhythmic segmentation even weak peristaltic movements can propel gut contents for long distances and cause excessive fluid to reach the colon and rectum. Thus, an ideal motility modifier for the control of diarrhoea should increase rhythmic segmentation but decrease peristalsis and intestinal secretions.

Opioids

Opioids have been used since antiquity to control diarrhoea, and they remain the mainstay of non-specific antidiarrhoeal therapy in humans. Several natural, semi-synthetic and synthetic opioids are available and are used also in domestic animals. The synthetic opioids like diphenoxylate and loperamide are preferably used in the treatment of diarrhoea than natural or semi-synthetic opioids because they penetrate poorly into the CNS and can produce antidiarrhoeal effects at doses which have minimal central effects.

Opioids increase intestinal tone and segmental activity, reduce propulsive movements, diminish intestinal secretions and enhance absorption of electrolytes and water. In humans, activation of μ receptors is associated with increased tone, activation of δ receptors reduces secretion, and activation of both μ and δ receptors appears to enhance electrolyte absorption. Direct action on intestinal smooth muscle and secretory/absorptive epithelium has also been demonstrated. Visceral pain is also suppressed by opioids. Although species differences have been demonstrated for site of action, receptor population and motility caused by opioids, these differences are more often quantitative rather than qualitative.

The widely accepted mechanism of opioids in the gastrointestinal tract is inhibition of acetylcholine release, perhaps by presynaptic inhibition, which in turn diminishes propulsive motility but enhances GI segmentation and tone (including that of sphincters). Intracellular mechanism may involve increased calcium dependent potassium conductance and hyperpolarisation. Opioids also reduce calcium entry during action potential and deplete neurons of calcium. Modulation of other endogenous mediators like serotonin is also likely to be involved in the opioid action.

Although opioids are effective in the treatment of moderate to severe diarrhoea, they should not be used in patients with chronic ulcerative colitis or acute bacillary or amoebic dysentery, since they appear to potentiate ulcerative processes in the colon and can provoke development of toxic megacolon.

Opium

Opium and its preparations are well known for their constipating action and have been used for many decades to treat diarrhoea. However, restrictions imposed by many countries on their use and availability of better synthetic opioids have limited their use. Various preparations of opium include powdered opium, tincture of opium (laudanum) and camphorated tincture of opium (paregoric). Tincture of opium contains 1 percent morphine and is often used in combination with kaolin. Camphorated tincture of opium (paregoric) contains 0.43% opium together with camphor, benzoic acid and oil of anise. Problems with opium or its preparations use include opiate dependency and analgesia which can mask symptoms of diseases which need treatment.

Dose

Tincture of opium (Tr. opii, laudanum)

Dogs : 0.3 – 1.3 ml (total), PO.

Cattle & horses: 15-60 ml (total).

Camphorated tincture of opium (Paregoric)

Dogs : 2 – 4 ml (total), PO, 3 times daily.

Foals & calves : 15 – 20 ml (total), PO.

Cattle & horses: 60 – 125 ml (total), PO.

Codeine

Codeine is an opium alkaloid that occurs as a colourless or translucent powder or crystals. It is primarily used as antitussive and sedative in dogs (Chapter 45). Its antidiarrhoeal effect is attributed primarily to its peripheral action on small intestine and colon, but it also has central effects.

Dose

Dogs : 0.25 – 0.5 mg/kg, PO, 3 to 4 times daily.

Diphenoxylate

Diphenoxylate is a synthetic piperidine opioid that is structurally related to pethidine. It is used specifically to control diarrhoea. It is the main active ingredient in the anti-peristaltic medication Lomotil, which also contains atropine.

Pharmacological effects

Diphenoxylate seems to act at the μ subtype of opioid receptors to slow intestinal contractions and peristalsis, thereby prolonging the transit time. This allows the intestine to remove moisture from the faecal contents and consolidate waste product into a dense solid form at a normal or higher rate. As an antidiarrhoeal, diphenoxylate is more potent than morphine. Though the antidiarrhoeal action is largely dependent on its peripheral effect on the GI wall, it can penetrate blood-brain barrier and may also produce systemic opiate effects.

Pharmacokinetics

After oral administration, diphenoxylate is rapidly absorbed and produces antidiarrhoeal and other systemic effects. It is rapidly de-esterified to difenoxin, an active metabolite that is eliminated with a half-life of 12 hours in humans.

Side effects/Adverse effects

Like other morphine derivatives, diphenoxylate may produce CNS stimulation in cats. In dogs, it may produce sedation due to its CNS depressant action. Other systemic opiate agonist effects may also occur in overdosage.

Clinical uses

Diphenoxylate is used clinically for the control of non-specific diarrhoea in small animals. In practice, diphenoxylate is available in combination with atropine. The atropine is added in non-pharmacological doses to discourage its abuse in human beings. Overdosage of combination produces unpleasant anticholinergic side effects such as dry mouth and blurred vision. The anticholinergic actions of the atropine may contribute to reduce intestinal motility and secretion, but these effects are not important.

Dose

Dogs : 0.1 – 0.2 mg/kg, PO, 2 to 3 times daily.
Cats : 0.05 – 0.1 mg/kg, PO, 2 times daily.

Difenoxin

Difenoxin (Motofen) is a 4-phenylpiperidine derivative and an active metabolite of diphenoxylate. Difenoxin has a high peripheral: central actions ratio, acting primarily on various opioid receptors in the intestines. Its pharmacological properties, adverse effects and uses are similar to those of diphenoxylate.

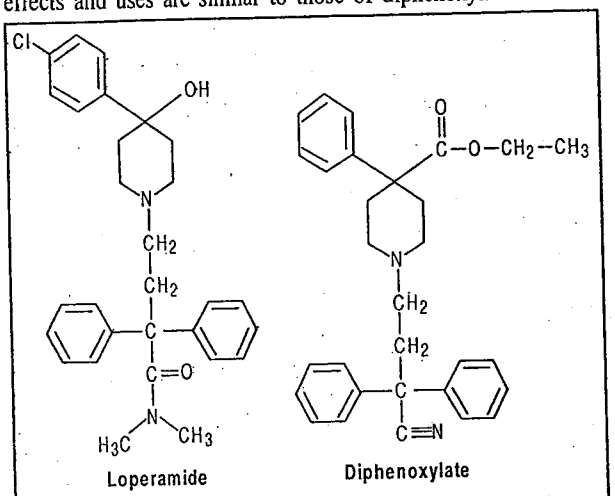


Fig. 43.8. Chemical structures of important opioid anti-motility drugs.

Loperamide

Loperamide is a synthetic piperidine derivative and an opioid that acts predominantly on μ-receptors in the gastrointestinal tract. It acts by decreasing the activity of myenteric plexus, which decreases the tone of the longitudinal smooth muscles but increases tone of circular smooth muscles of the intestinal wall. This allows intestine to absorb more water out of the faecal matter. Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex. It also produces antisecretory effects due to its direct interaction with calmodulin (blocks calmodulin induced secretion).

Pharmacological effects

Loperamide is 40 to 50 times more potent than diphenoxylate and has fewer side effects. It has also a longer duration of action than diphenoxylate. Because of its poor water solubility, loperamide absorption after oral administration is very less. Unlike diphenoxylate, it cannot penetrate blood-brain barrier in significant concentration; so CNS effects are negligible in normal dosage.

Side effects/Adverse effects

Serious side effects are rare with loperamide. Overdosage may cause prostration, ataxia, vomiting, hypersalivation, bradycardia or hypothermia. Rare side-effects associated with loperamide are paralytic ileus and rashes. Like diphenoxylate, it should be used with care in cats.

Contraindications and precautions

Loperamide and other antimotility drugs are contraindicated in animals with invasive or toxigenic intestinal bacterial infections because they delay clearance of pathogens or toxins from the intestine. These drugs should not to be used indiscriminately in puppies with parvoviral enteritis. Antimotility drugs like loperamide should not be used in severe colitis as they may lead to toxic megacolon. Loperamide is not recommended for use in hepatic failure since it can precipitate hepatic encephalopathy.

Clinical uses

Loperamide is useful in the symptomatic control of acute and chronic non-specific diarrhoea. It can be used for diarrhoea, when there is grossly excessive loss of water and electrolyte. Loperamide has also been used to ameliorate diarrhoea in patients with stool bowel syndrome. Loperamide has poor penetration into CNS, so is acceptable in cats.

Dose

Dogs : 0.1 – 0.2 mg/kg, PO, 2 to 3 times daily.
Cats : 0.08 – 0.16 mg/kg, PO, 2 times daily.

3. Antisecretory and Anti-Inflammatory Agents

Several drugs with antisecretory and anti-inflammatory actions are employed in the treatment of diarrhoea in both human and veterinary practices.

a. Salicylic Acid Derivatives

Sulphasalazine

Sulfasalazine (Sulicylazosulphapyridine) is a 5-aminosalicylic acid (5-ASA) joined to sulphapyridine by a covalent bond (Figure 43.9). It is considered useful for the treatment of inflammatory bowel disease and colitis in small animals.

Mechanism of action and Pharmacological effects

Sulphasalazine has slow solubility, so is minimally absorbed by the small intestine. When the unabsorbed sulphasalazine reaches colon, its azo bond is hydrolysed by the colonic bacteria to release 5-aminosalicylic acid (5-ASA) and sulphapyridine. The salicylate (5-ASA) part inhibits synthesis of cyclooxygenase, thromboxanes synthase and interleukin 1, as well as production of reactive oxygen species and is responsible for the local anti-inflammatory effect. The clinical effect produced by 5-ASA decreases mucosal secretion and affords considerable relief in ulcerative colitis and related inflammatory bowel diseases. The beneficial effect of sulphasalazine is not due to any antibacterial action produced by sulphapyridine, whose function is only to carry 5-ASA to the colon without being absorbed proximally.

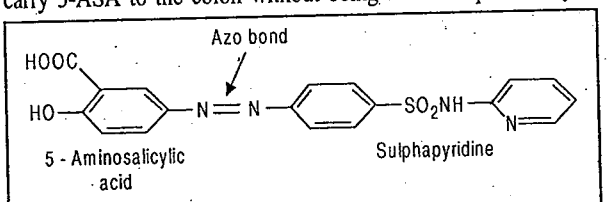


Fig. 43.9. Chemical structure of sulphasalazine

Pharmacokinetics

The sulphasalazine remains largely unabsorbed in the GI tract after oral administration. However, a part of released sulphapyridine and a small percentage of 5-ASA is absorbed in the colon. The absorbed sulphapyridine and 5-ASA are metabolised in liver and are then excreted in urine.

Side effects/Adverse effects

The common side effects of sulphasalazine are anorexia and vomiting. Keratoconjunctivitis sicca may occur rarely with prolonged use and is often irreversible. The side effects have been attributed primarily to the absorbed sulphapyridine moiety, but are also occasionally seen with drugs containing 5-ASA only. In laboratory

animals, high doses of sulphasalazine have produced infertility in males, which was found to be reversible on discontinuation.

Contraindications and precautions

Sulphasalazine is contraindicated in animals with intestinal or urinary obstructions. It should be used with caution in animals with pre-existing liver, renal or haemolytic disease. Care should be taken with administration to cats, as cats are more sensitive to salicylates.

Clinical uses

Sulphasalazine is used mainly for the treatment of inflammatory bowel disease in dogs and cats. It is also considered useful for treatment of chronic colitis. It is also effective in several types of arthritis, particularly rheumatoid arthritis.

Dose

Dogs : 10 – 30 mg/kg, PO, 3 times daily. Dose may be increased up to 50 mg/kg, if required.

Cats : 10 – 20 mg/kg, PO, once daily.

Mesalazine

Mesalazine (Mesalamine) is the official name given to 5-aminosalicylic acid (5-ASA). In mesalazine, the 5-ASA is encased in an enteric coated formulation, which releases it at pH > 6 (i.e. in mid-small intestine). This way 5-ASA is required in lower amount than that is present in sulphasalazine and is without side effects. Coated mesalazine is also better tolerated than sulphasalazine. After oral administration, less than half of 5-ASA is absorbed, acetylated in liver and excreted in urine. Mesalazine is contraindicated in renal and hepatic impairment

Mesalazine is formulated for oral ingestion as tablets or granules, and for rectal administration as a rectal suppository and suspension. Mesalazine enemas are also available, which may be given as retention enemas. This is inconvenient, but can be exceptionally effective for animals with severe inflammation that requires immediate control.

Olsalazine

Olsalazine consists of two molecules of 5-ASA coupled together by an azo bond. Unlike mesalazine, it does not need any separate carrier moiety to deliver the drug at the required site. Similar to sulphasalazine, it is poorly absorbed in the ileum and the azo bond splits in the colon to provide 5-ASA locally. Olsalazine is considered a more reliable preparation for delivery of 5-ASA to large intestine, which is the active site of disease in ulcerative colitis. However, it often aggravates diarrhoea initially by decreasing transit time through bowel. Its indications, contraindications and side effects are similar to those of sulphasalazine, but chances of development of keratoconjunctivitis sicca are much lesser than sulphasalazine.

Dose

Dogs : 10 – 20 mg/kg, PO, 2 times daily.

Balsalazide

Balsalazide is 5-aminosalicylic acid linked to 5-aminobenzoyl-β-alanine as the carrier. The 5-ASA is released in the colon and the carrier is largely excreted unabsorbed. Balsalazide is used in the treatment of inflammatory bowel disease and ulcerative colitis.

B. Anticholinergics

Several antimuscarinic drugs diminish secretory and motor activity of the GI tract. These drugs include natural belladonna alkaloids (e.g. atropine and hyoscine), semi-synthetic drugs (e.g. methylatropine nitrate, methylhyoscine bromide, butylhyoscine bromide, anisotropine methylbromide), and synthetic anticholinergic agents (e.g. aminopentamide, dicycloverine, glycopyrronium, mepenzolate, oxyphenonium, propantheline, benzetimide, pipenzolate, clidinium and camylofine). They often decrease tone and propulsive movement of intestine and relax spasm of visceral smooth muscles. Accordingly, they are also known as antispasmodics or spasmolytics.

Anticholinergic drugs provide symptomatic relief from non-specific diarrhoea by reducing gut secretion and motility. The main benefit of anticholinergic agents may be related to their ability to reduce intestinal secretions rather than intestinal motility. They may benefit nervous or drug (e.g. neostigmine and metoclopramide) induced diarrhoea. Combinations of anticholinergics drugs (e.g. clidinium or isopropamide) with tranquillizer/neuroleptic drugs (e.g. prochlorperazine or chlordiazepoxide) are useful in stress-related or psychogenic diarrhoeas. Possible side effects of anticholinergics may include xerostomia, urinary retention, constipation, tachycardia and CNS stimulation. The synthetic groups are mostly substituted quaternary amines and are devoid of central effects. The pharmacology of anticholinergic drugs has been described in detail in Chapter 14.

C. Miscellaneous Anti-Secretory Drugs

A number of non-specific drugs have shown some antidiarrhoeal activity. However, these drugs have not been extensively studied and are not routinely used for the treatment of diarrhoea.

Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indometacin and flunixin inhibit cyclooxygenase pathway and block prostaglandins synthesis. Though the roles of various prostaglandins in intestinal motility as well as in absorption and secretory processes are complex, yet some acute and chronic diarrhoeal syndromes respond well to NSAIDs. These drugs may also be of value in ameliorating intestinal inflammation and reducing hypersecretion in neonatal calf diarrhoea. NSAIDs should not be used in hypovolaemic patients due to risk of inducing renal failure.

Glucocorticoids

Glucocorticoids like prednisolone, hydrocortisone, prednisone, betamethasone, dexamethasone and tixocortol have been found to be beneficial in treating refractory chronic diarrhoeal diseases as well as chronic inflammatory diseases of the GI tract. Glucocorticoids stimulate active sodium absorption in jejunum, ileum and colon. As glucocorticoids produce undesirable side effects after prolonged use, they should be replaced by other drugs as soon as possible. The detailed pharmacology of glucocorticoids is given in Chapter 51.

Adrenergic Agonists

Adrenergic agonists at low concentration increase basal fluid absorption. The α_2-adrenoceptor agonist clonidine can reduce intestinal smooth muscle tone and secretory activity and has been found to be helpful in treating diarrhoea caused by *E. coli*, cholera and diabetes.

Calcium-Calmodulin Antagonists

Calcium-calmodulin antagonists may act by stimulating active absorption as well as by inhibiting stimulated intestinal secretion. Chlorpromazine has a marked intestinal ability to bind calmodulin, which probably interfere with the effect of cAMP in secretory

process. Chlorpromazine, trifluperazine and some other drugs are thus useful in the control of secretory diarrhoea. These drugs are associated with unacceptable sedation in animals.

Octreotide

Octreotide is an octapeptide that mimics natural somatostatin pharmacologically as it contains a portion of the somatostatin molecule (amino acids 7-10). Somatostatin is suggested to be involved in the inhibition of gastric acid and pepsinogen secretion, inhibition of endocrine secretions (e.g. gastrin, cholecystokinin, secretin, vasoactive intestinal peptide and motilin), inhibition of intestinal fluid and bicarbonate secretion, and diminution of smooth muscle contractility. However, its plasma half-life is very short (about 2 minutes) to be useful as a drug. Octreotide is as potent as somatostatin but has longer plasma half-life of 90 minutes in man.

Octreotide has been used in human medicine to control diarrhoea related to carcinoid and vasoactive intestinal peptide (VIP) secretory tumours, and also refractory diarrhoea in AIDS patients. It is also useful in various motility disorders and gastrointestinal bleeding. Adverse effects of short-term therapy are generally minor and include nausea and gastrointestinal upset. The drug has to be given by parenteral (SC) route. The clinical data on octreotide in veterinary species are lacking.

Racecadotril

Racecadotril (Acetorphan) is a peripherally acting inhibitor of enkephalinase, the enzyme that degrades endogenous enkephalin opioid peptides. By decreasing the metabolic inactivation of peripheral endogenous enkephalins, racecadotril appears to enhance the action of naturally occurring opioids on the GI tract. This produces an antidiarrhoeal action. Unlike other opioid drugs used to treat diarrhoea, which reduce intestinal motility, racecadotril has mainly an antisecretory effect. Racecadotril is often used as an adjunct to oral rehydration therapy to reduce the duration and volume of watery diarrhoea.

4. Miscellaneous Antidiarrhoeal Drugs

Intestinal Astringents

Astringents are substances which reversibly precipitate proteins, alter surface characteristics and tend to form a protective layer on the mucosal surface. The precipitation of proteins are on the surface only and underneath the cells are viable. The precipitated proteins act as a barrier between tissue and irritant and the underlying tissue is therefore soothed and protected from further damage. The precipitation of proteins also does not allow absorption of toxins, movement of fluid in either direction, and may decrease pain, if some exposed nerve is affected. This way, they also help to decrease gut motility. However, astringents are nowadays not used frequently to control diarrhoea because many of these agents are caustic and potentially toxic (e.g. metallic astringents). Astringents also interfere with absorption of nutrients, electrolytes and water.

Various compounds possessing astringent action include vegetable (e.g. tannic acid) and metallic (e.g. sulphates and hydrochlorides of zinc, copper, aluminium and iron) astringents. Most frequently used are vegetable astringents and catechu.

Tannic acid

Tannic acid (Gallotannic acid) is a specific commercial form of tannin, a type of polyphenol acting as astringent. It occurs as a complex glycoside mixture in combination with glucose. The chemical formula for commercial tannic acid is often given as $C_{76}H_{52}O_{46}$. Tannic acid is usually extracted from gallnuts from *Rhus semialata* (Nut gall) or *Quercus infectoria* (Oak gall) trees. It is also found in some other plants, but the concentration is very high in oak gall. Tannic acid obtained from the oak galls is often called gallotannic acid to differentiate it from other sources of tannic acid. Tannic acid is a light amorphous powder, which is readily soluble in water and alcohol.

After oral administration, tannic acid is released from the glycosidic bond and it acts rapidly and locally to produce astringent action. However, the action is only of short duration as it is rapidly metabolised further to gallic acid and pyrogallic acid, which do not have astringent action. The rate of hydrolysis of glycosides varies with different plant sources. Tannic acid is not absorbed as such from the GI tract, but gallic acid is absorbed and metabolised further in the body.

Several commercial preparations contain tannic acid. Although tannic acid is reported to reduce the severity of diarrhoea, its effectiveness is questionable. Tannic acid in overdosage may cause GI irritation and hepatic centrilobular necrosis.

Dose

Dogs	:	0.6 g (total), PO, daily.
Sheep & goats	:	2.6 g (total), PO.
Cattle & horses	:	Up to 24 g (total), PO.

Catechu

Catechu (Hindi: *Katha*) is an extract of any of several species of *Acacia*, but especially *Acacia catechu*. It contains catechutannic acid (22-50%), a catechol tannin that accounts for its astringent action. Catechu is preferable to tannic acid (gallotannic acid) because the slow release of catechutannic acid decreases its toxicity and increases duration of action. Catechu also affects the large intestine where it reaches in unchanged form to a significant amount.

Dose

Dogs	:	0.3 – 1 g (total), PO.
Horses	:	4 – 9 g (total), PO.
Cattle	:	8 – 24 g (total), PO.

Other drugs

Carboxymethylcellulose, psyllium and polycarbophil are fibre supplements used primarily for constipation, but they can increase the viscosity of faeces in diarrhoea and give the perception of improvement. They may be used in irritable bowel and ileostomy/ colostomy diarrhoea. These agents are not useful in acute diarrhoea.

II. SPECIFIC CHEMOTHERAPEUTIC AGENTS

Several classes of chemotherapeutic agents are employed for the treatment of infectious diarrhoea. These include various antibacterial drugs like aminoglycosides (e.g. neomycin, streptomycin and spectinomycin), penicillins (e.g. ampicillin and amoxicillin), sulphonamides (e.g. succinylsulphathiazole and phthalylsulphathiazole), nitrofurans (e.g. nitrofurazone and furazolidone) and quinolones (e.g. ciprofloxacin and enrofloxacin). Clinical use of antifungal (e.g. miconazole), antigiardial (e.g. metronidazole and dimetridazole) and anthelmintic (e.g. albendazole and fenbendazole) may also be required in appropriate conditions.

The choice of antibacterial drugs in made on the basis of spectrum of activity and characteristics of absorption. Antibacterial agents which are poorly absorbed from the GI tract may preferably

be used. However, antibacterials which are absorbed systemically are particularly indicated in acute haemorrhagic diarrhoea and where there is evidence of bacterial invasion of the intestinal mucosa. These drugs should be indicated when there is severe mucosal damage, notable inflammatory reaction (acute or chronic), systemic involvement from the bacterial enteritis or hepatic encephalopathy. If the diarrhoea is not due to primary or secondary bacterial infection, antibacterial are unlikely to be useful. Antibacterial are not useful in diarrhoeas caused by viral, fungal or protozoal infection. Use of chemotherapeutic agents has some limitations like they can alter normal intestinal flora, prolong carrier state and allow superinfection to emerge.

REPLENISHMENT OF FLUID AND ELECTROLYTE LOSS

Rehydration therapy with oral or intravenous solution is often life saving in most types of diarrhoea because deaths following diarrhoea often result from dehydration. In majority of cases, oral fluids will suffice but severe diarrhoea may require parenteral replenishment of fluid and electrolyte, especially in patients unable to take fluid by mouth.

Oral Rehydration Solutions

Oral rehydration solutions (ORS) essentially containing Na^+, K^+, Cl^- and glucose are widely used in man and small animals to correct water and electrolyte loss due to diarrhoea and some other conditions. These solutions are normally made isotonic (300 mOsm/kg) because absorption of water from the intestinal lumen occurs only when an isotonic state is reached. Glucose is an important component of the ORS because it facilitates absorption of sodium. The molar ratio of glucose in the ORS is usually kept somewhat higher than Na^+ because excess glucose is utilised for absorbing the Na^+ present in intestinal secretions in addition that present in ORS itself. Glucose-facilitated Na^+ absorption continues to occur in diarrhoea even when other mechanisms have failed or when intestinal secretion is excessive. Further secreted fluid lacks glucose and, therefore, cannot be reabsorbed. Potassium is also an important constituent of ORS because in most diarrhoeas, the K^+ loss is substantial. The base (bicarbonate, citrate or lactate) is often added to ORS to correct acidosis due to alkaline (HCO_3^-) loss in the faeces. Base free ORS has been found to be equally effective in rehydration, though correction of acidosis is slower.

Though the composition of ORS varies according to the type of diarrhoea, a universal formula recommended by the WHO (Table 43.3) for humans is also used in small animals. This formula provides Na^+ 90 mM, K^+ 20 mM, Cl^- 80 mM, citrate (base) 30 mM, and glucoses 110 mM.

Super ORS: This is a solution that in addition to rehydration leads to decrease in purging rates and improvement of diarrhoea by enhancing absorption. For this, certain actively transported amino acids (e.g. alanine and glycine) which co-transport Na^+ has been tried. The efficacy of super ORS is marginal and it also is costly.

Intravenous Rehydration Solutions

Intravenous rehydration is needed only when fluid loss is severe (i.e. >10% body weight), or if the animal is losing fluid >10 ml/kg/hour or is unable to take enough oral fluids due to weakness, recumbency or vomiting. Although various intravenous rehydration fluids are available and can be used in diarrhoeas (See Chapter 40), Ringer lactate is considered a good rehydration fluid. In most cases, oral rehydration could be instituted after the initial parenteral volume replacement.

Table 43.3: Composition of oral rehydration solution.

Compound	Concentration (mM)	Concentration (g/L)
Sodium chloride	60	3.5
Potassium chloride	20	1.5
Sodium citrate	30	2.9
Glucose	110	20.0

MAINTENANCE OF NUTRITION

Contrary to traditional view, the patients of diarrhoea should not be starved. Fasting causes villous atrophy, decreases brush border disaccharidase enzymes, and reduces absorption of water, electrolytes and nutrients. Fasting may lead to malnutrition, if diarrhoea is prolonged or recurrent. Feeding during diarrhoea has been shown to increase intestinal digestive enzymes and cell proliferation in mucosa. However, in practice many veterinarians follow withholding of food for 24 hours in dogs and cats, and provision of oral electrolyte replenishment solutions. This is followed by a bland diet and gradual re-introduction to normal feed. Hypertonic oral solutions are available which minimise the deleterious effects on gut structure and energy balance caused by withholding food or milk for a short time.

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CHAPTER 44 DRUGS AFFECTING EMESIS AND OTHER FUNCTIONS OF DIGESTIVE SYSTEM

- Drugs affecting emesis
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DRUGS AFFECTING EMESIS

Nausea and emesis (vomiting) are the symptoms of serious organic disturbances involving any of the viscera of chest or abdomen, or are produced by drugs, radiation, motion sickness, infections, metabolic and emotional disturbances, neoplasm and painful stimuli. Clinically, emesis is induced by use of drugs in order to empty the anterior portion of digestive tract in cases of intoxication by ingestion of non-corrosive poisons. Vomiting is usually considered a protective reflex that occurs effectively only in certain species of animals like carnivores and omnivores. Horses, ruminants, rodents, guinea pigs and rabbits are unable to vomit.

Mechanisms of Emesis

Emesis is a complex process that occurs due to stimulation of vomiting (emetic) centre situated in the medulla oblongata. Although several afferent pathways and some higher centres in brain may be responsible for initiating emesis, the emesis primarily occurs via the

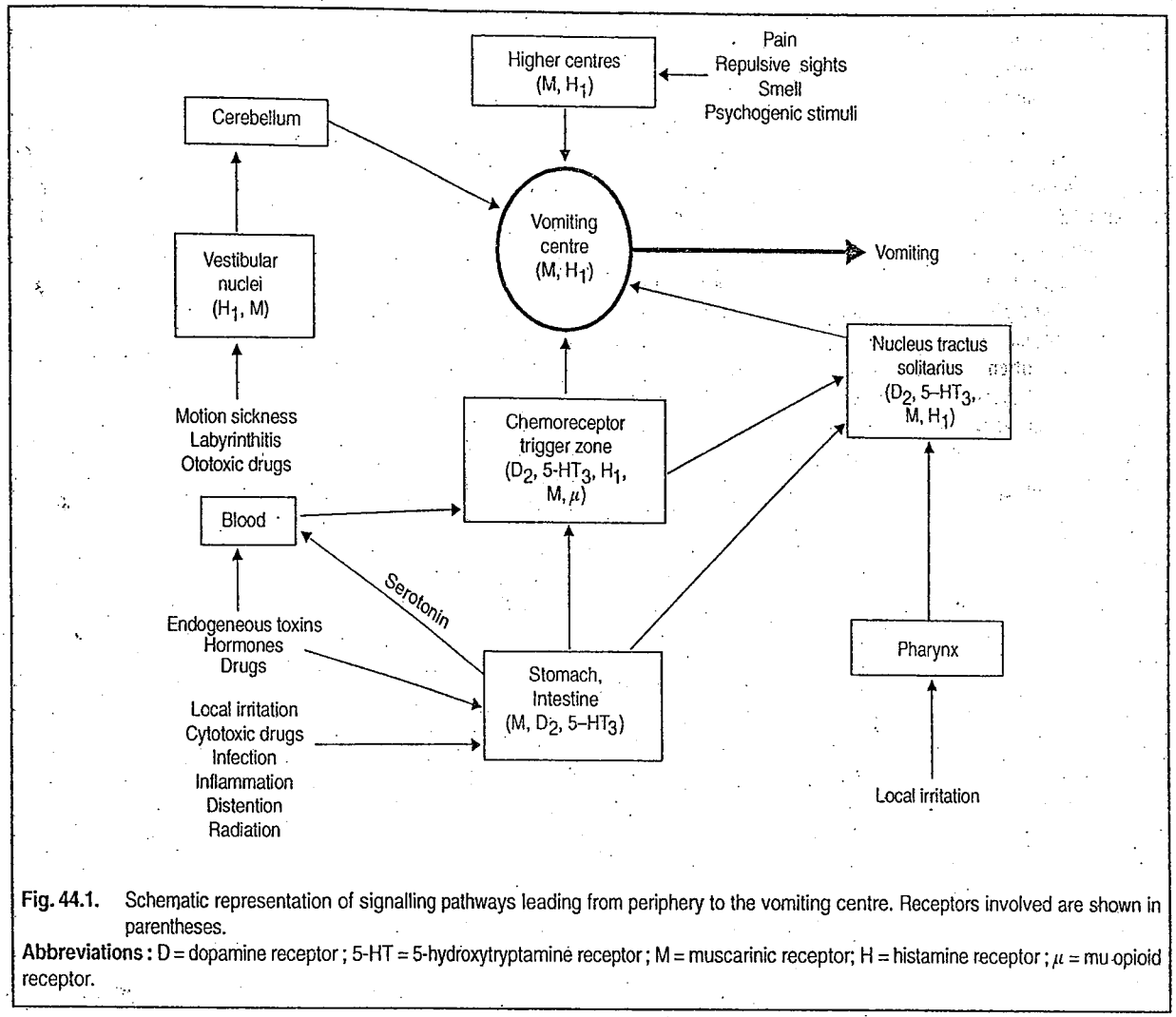


Fig. 44.1. Schematic representation of signalling pathways leading from periphery to the vomiting centre. Receptors involved are shown in parentheses.

Abbreviations : D = dopamine receptor ; 5-HT = 5-hydroxytryptamine receptor ; M = muscarinic receptor; H = histamine receptor ; μ = mu-opioid receptor.

emetic/vomiting centre in medulla that coordinates all signals. Various pathways involved in process of emesis include:

- Impulses to the [redacted] in medulla may arise from higher centres such as cerebral cortex and limbic system. Visual and olfactory stimuli leading to vomiting originate in the cerebral cortex, whereas those from increased intracranial pressure and head injury originate via the limbic system. [redacted]
- Impulses originating from the semicircular canal of [redacted] are transmitted by the [redacted] to vestibular nuclei and then via cerebellum to the vomiting centre. Muscarinic and histaminergic synapses seem to be involved in this pathway causing vomiting due to motion sickness and labyrinthitis (Figure 44.1).
- Peripheral afferent pathways include those arising from stimulation of [redacted] Impulses may be carried by sympathetic or vagal afferents from heart, stomach, small intestine, liver, gall bladder, peritoneum, kidney, ureter, urinary bladder, uterus, testes and some other visceral organs. [redacted] In stomach, D_2-dopamine receptors appear to inhibit gastric motility during nausea and vomiting and also allow relaxation of upper stomach and delayed emptying associated with gastric distension caused by food. Serotonin also mediates in the afferent pathways from the stomach and small intestine.
- d. [redacted] This area does not possess complete blood-brain barrier so is more readily accessible to foreign substances present in the circulating blood and cerebrospinal fluid. The CTZ alone cannot induce vomiting but acts through vomiting centre to which it is attached through neural pathways (Figure 44.1). Emesis caused by blood borne mediators associated with certain diseases (e.g. uraemia, pyometra, liver disease, endotoxaemia and radiation sickness disease) and drugs (e.g. digitalis, apomorphine, opioid analgesics and oestrogens) is mediated through CTZ. [redacted] Some peripheral signals bypass the trigger zone, reaching the vomiting centre via NTS.

The CTZ and NTS express a variety of receptors viz. H_1-histamine, muscarinic, D_2-dopamine, 5-HT_3-serotonin and μ-opioid

receptors. The vomiting centre in the brainstem also contains high concentrations of substance P and its receptor, which appears to regulate vomiting. Alpha$_2$-adrenoceptor associated with the area postrema also induces emesis in dogs and cats. Cytotoxic drugs and GI irritants release serotonin from enterochromaffin cells, which either acts on 5-HT$_3$ receptor present on vagal afferents and sends impulses to CTZ and NTS or spills into circulation that reaches CTZ. Serotonin may also be released from platelets by inflammatory mediators.

Following stimulation of the vomiting centre, various efferent pathways including vagus, phrenic nerves and spinal innervations of the abdominal musculature mediate emesis. The initial manifestations often involve nausea accompanied by reduced gastric tone and peristalsis. In the emetic response, fundus and body of stomach, oesophageal sphincters and oesophagus relax, while duodenum and pyloric stomach contract in the retrograde manner. Rhythmic contractions of diaphragm and abdomen then compress the stomach leading to expulsion of gastric contents through mouth.

EMETICS

Emetics are drugs which are used to evoke vomiting. Clinically, emesis may be induced to empty the anterior portions of the digestive tract either [redacted] or when a non-corrosive poison has been ingested. Emetics are employed [redacted] rarely in pigs, and not in horses and cattle. They should be administered only to animals capable of vomiting.

Classification

Broadly, emetics can be divided into main groups - locally acting and centrally acting emetics.

[redacted]

e.g. ipecacuanha, luke warm water, sodium chloride, sodium carbonate, copper sulphate, zinc sulphate and hydrogen peroxide.

[redacted]

e.g. apomorphine and xylazine.

I. Locally Acting/Reflexly Acting Emetics

[redacted] Distension of these organs also contributes to the initiation of emetic reflex. Some of the useful agents of this group include simple household substances:

Ipecacuanha

Ipecacuanha (Ipecac) is obtained from dried roots and rhizomes of plant [redacted] Ipecacuanha syrup, prepared from powdered ipecacuanha, is a [redacted] Ipecacuanha is available commercially as a clear to amber coloured hydrochloride ipecacuanha syrup having a characteristic odour. Each ml of ipecacuanha syrup contains 70 mg of powdered ipecacuanha. Ipecacuanha liquid extract contains 2% of emetine, the active constituent present in ipecacuanha.

Mechanism of action

Ipecacuanha contains active alkaloids emetine (methylcephaeline) and cephaeline, which are believed to cause the major pharmacological effects of the drug. Ipecacuanha acts both locally by irritating the gastric mucosa and centrally by stimulating the chemoreceptor trigger zone.

Pharmacological effects

After oral administration of ipecacuanha to dogs and cats, vomiting usually occurs within 15-30 minutes. However, its effectiveness is unpredictable. The pharmacokinetic data on ipecacuanha in domestic animals are scarce.

Side effects/Adverse effects

At recommended doses ipecacuanha syrup rarely exhibits toxic effects, but it can induce lachrymation, salivation and bronchial secretions. At high doses, it may result in serious cardiotoxicity with resulting arrhythmias, hypotension or fatal myocarditis.

Treatment: No specific antidote therapy is available to ipecacuanha toxicity. Activated charcoal may be given to adsorb the unabsorbed ipecacuanha. Supportive therapy may also be employed.

Contraindications and precaution

In addition to various conditions limiting use of emetics, ipecacuanha syrup should not be used in animals with pre-existing cardiac dysfunction. Administration of ipecacuanha with milk, dairy products, carbonated beverages or activated charcoal should be avoided as ipecacuanha efficacy may be diminished. Ipecacuanha fluid extract should not be used in place of ipecacuanha syrup, as fluid extract is about 10-14 times more potent than syrup and may result in cardiotoxicity and death. If the animal has nearly empty stomach, 5ml/kg of water should preferably be given immediately after ipecacuanha administration.

Dose

Dogs & cats : 1 – 2 ml/kg, PO. Total dose should be more than 15 ml for a dog. Drug may be repeated in 20 minutes, if emesis does not occur. If emesis failed to occur even after the second dose, gastric lavage should be performed to remove excess of ipecacuanha.

Luke-warm water/Tepid water

Luke warm water administered by a stomach tube is helpful in inducing vomiting in case of a non-corrosive substance poisoning. In such a case water may dilute the poison and may also induce vomiting either by distension of stomach or by mild irritation.

Sodium chloride

Salt water has been used since ancient times as emetic. Sodium chloride either in the crystalline form or as a saturated or strong tepid water solution may produce emesis in 10-20 minutes. Half to one teaspoonful of plain table salt placed in the pharyngeal region on the back of tongue may also induce emesis in dogs. Care must be taken with salt, as excessive intake can potentially be harmful.

Dose

Saturated sodium chloride solution

Dogs : 30 – 60 ml, PO, depending on the size of animal.

Sodium carbonate

Sodium carbonate, also known as washing soda or soda ash, is a sodium salt of carbonic acid. A large sodium carbonate crystal placed on the back of tongue may induce vomiting. However, the sodium carbonate is less reliable than sodium chloride salt as an emetic.

Copper sulphate

Copper sulphate is an irritant. One percent solution of copper sulphate given orally to dogs may induce vomiting in 30-45 minutes through irritation of GI tract. However, copper sulphate solution is not a reliable emetic. Further, presence of any undissolved crystals of $CuSo_4$ in the GI tract may act as caustic and cause damage to GI epithelium. It also produces metallic taste in mouth and burning pain in chest and abdomen. Copper sulphate is now less used to induce emesis mainly due to its toxicity.

Dose

Copper sulphate 1% solution

Dogs : 10 – 30 ml, PO, depending on the size of animal.

Zinc sulphate

Zinc is a naturally occurring mineral, considered a useful nutrient added in animal feeds. Like copper sulphate, one percent solution of zinc sulphate can be used to induce vomiting in small animals. Similar to copper sulphate, emesis produced by zinc sulphate in less reliable.

Dose

Zinc sulphate 1% solution

Dogs : 10 – 30 ml, PO, depending on the size of animal.

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is an oxidizer commonly used as a bleach. Medically it is used as a disinfectant and antiseptic due to its oxidizing properties. Oral administration of hydrogen peroxide (3%) induces rapid emesis in cats and dogs due to irritation of the GI tract. Hydrogen peroxide is clear liquid, slightly more viscous than water; therefore fatal aspiration of hydrogen peroxide foam is possible.

II. Centrally Acting Emetics

Centrally acting emetics directly stimulate the vomiting centre or chemoreceptor trigger zone in brain. Although many drugs may cause vomiting through central mechanisms, only a few drugs are used clinically for this purpose.

Apomorphine

Apomorphine is a semi-synthetic derivative of morphine, but it does not actually contain morphine or its skeleton, or bind to opioid receptors. It occurs as a white powder or greyish white or colourless glistening crystals which on exposure to air and light attains a greenish tint. Apomorphine solutions are more stable in acidic than alkaline solutions. A 0.3% solution of apomorphine has a pH of about 3-4.

Mechanism of action

Apomorphine is a non-selective dopamine agonist which activates both D_1-like and D_2-like receptors, with some preference for the latter subtypes. By acting on central dopamine receptors, apomorphine stimulates dopaminergic neurons in the chemoreceptor trigger zone to produce vomiting.

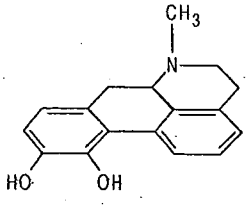


Fig. 44.2. Structure of apomorphine

Pharmacological effects

Apomorphine is a potent emetic. It induces emesis in 3-10 minutes following subcutaneous or conjunctival administration. The emesis may continue for 5-15 minutes, which is being followed by exhaustion and depression. Apomorphine can cause both CNS depression and stimulation, but it tends to produce more stimulatory effect in cats in which it should not be used. Medullary centres can be depressed with resultant respiratory depression, especially in high doses.

Pharmacokinetics

Apomorphine can be administered by almost any route to cause vomiting. However after oral administration, it is absorbed slowly, required in larger doses, and has unpredictable efficacy. Therefore, it is often administered by parenteral route or applied topically to the eye. After IM or SC administration, apomorphine produces vomiting within 5 minutes. Topical administration to the conjunctival sac is usually effective, but it is less useful than parenteral administration. After absorption, apomorphine is primarily conjugated in the liver and then excreted in the urine.

Side effects/Adverse effects

At therapeutic doses, apomorphine may produce protracted vomiting. Overdoses of apomorphine may result in respiratory and/or cardiac depression through opioid receptors. Central nervous system stimulation (excitation and seizures) with respiratory centre depression may occur in toxic doses.

Treatment: Naloxone may reverse the CNS and respiratory effects of apomorphine because they are mediated through opioid receptors, but it cannot halt vomiting that occurs via dopamine receptor stimulation. Atropine can be used to treat severe bradycardia.

Contraindications and precautions

In addition to general contraindications to vomiting, apomorphine should not be used in presence of existing central depression as it may aggravate respiratory and CNS depression. If vomiting does not occur within the expected time after apomorphine administration, repeated doses are also unlikely to induce emesis and may cause symptoms of toxicity because emetic centre is depressed like other medullary centres. Apomorphine does not remain stable for more than 24 hours in a plastic container, so syringes and containers are discarded if not used within 24 hours. Apomorphine is colourless as a liquid but stains green. The discoloured tablets or solution with emerald green colour should not be used.

Drug interactions

Antiemetic drugs, particularly antidopaminergic drugs (e.g. phenothiazines), may negate the emetic effect of apomorphine. Apomorphine should not be used along with CNS depressant drugs like opioids and barbiturates.

Clinical uses

Apomorphine is used as an emetic primarily in dogs, in which it is considered to be the emetic of choice. However, it is not much effective in pigs and should not be recommended in cats. If a cat must be made to vomit, one may cautiously use apomorphine at the same dose rate as in the dog or preferably employ xylazine.

Dose

Dogs : 0.04 mg/kg, IV (preferred route).
: 0.04 – 0.08 mg/kg, IM or SC.
: 0.25 mg/kg into the conjunctival sac. Alternately, half or whole of soluble table (6 mg) is crushed and dissolved in few drops of water and administered into the conjunctival sac. After sufficient vomiting, conjunctival sac should be rinsed free of apomorphine.

Xylazine

Xylazine is a potent alpha$_2$-adrenergic agonist and a sedative/analgesic with muscle relaxant properties. Xylazine is also an effective and reliable emetic when administered to cats, but it is less effectious in dogs. While thought to be centrally mediated, neither dopaminergic blockers (e.g. phenothiazines) nor alpha-adrenoceptor blockers (e.g. tolazoline and yohimbine) block the emetic effect of xylazine. Vomiting is generally seen within 3-5 minutes after parenteral administration of xylazine in cats and occasionally in dogs. Emesis is usually followed by sedation.

Important adverse effects in dogs and cats include muscle tremors, bradycardia, reduced respiratory rate, movement in response to sharp auditory stimuli, and increased urination in cats. Seizures have been reported after overdoses. The pharmacology of xylazine has been discussed in detail in Chapter 21.

Dose

As antiemetic

Cats : 0.4 – 1 mg/kg, IM.

Contraindications to emetics

All emetics are contraindicated in following conditions:

a. *Corrosive (e.g. acid and alkali) poisoning:* Emetics should not be used in corrosive poisoning due to enhanced risk of perforation and more injury to oesophageal and buccal mucosa.

b. *Unconscious/comatose patient:* Emetics should not be used in comatose or unconscious patients because of risk of aspiration of vomitus due to impaired pharyngeal and laryngeal reflex.

c. *Oesophageal obstruction:* Emetics should not be used in oesophageal obstruction because increased pressure may cause damage/rupture of stomach and intestine.

d. *Late poisoning:* Emetics should not be used in advanced stage of poisoning because they may produce or aggravate shock.

e. *Ingestion of foreign objects:* Emetics should not be used when pointed or sharp foreign substances have been ingested. Sharp objects may perforate GI tract.

f. *Petroleum products:* Emetics should not be used in case of intoxication by petroleum distillate (e.g. kerosene and gasoline) due to more chances of aspiration of liquid of low viscosity.

g. *CNS excitation:* Emetics should not be used in case of poisoning by CNS stimulants (e.g. strychnine) or seizures because convulsions are often precipitated by emetics.

h. *CNS depression:* Emetics should not be used in case of existing CNS depression or poisoning by CNS depressants because centrally acting emetics like apomorphine often precipitate CNS depression.

i. *Species lacking vomiting mechanism:* Emetics should not be used in species like horses, ruminants, rodents and rabbits because these animals are either unable to vomit or do not have stomach walls strong enough to tolerate the act of emesis. Further, emetics may produce toxicosis in these species.

j. *Geriatric and weak patients:* Emetics should not be used in old or extremely weak patients because such patients cannot tolerate act of emesis.

k. *Pregnancy:* Emetics should not be used in pregnant animals due to chances of abortion.

l. *Pathological conditions and surgery:* Emetics should not be used in conditions like prolapse, hernias or recent abdominal surgery because emesis may aggravate the existing conditions or may rupture the sutures in surgery.

ANTI-EMETICS

Antiemetics are drugs which are used to prevent or suppress vomiting. Vomiting occurs frequently as a complication of several systemic or local disease conditions and may be extremely detrimental for a patient. Protracted vomiting leads to dehydration, acid-base and electrolyte disturbances and ultimately physical exhaustion. Fatigue, aspiration pneumonia and gastric rupture are also potential complications. Debilitating vomiting may also occur as a side effect following radiation therapy and cancer chemotherapy. Considering the complexity of the mechanisms involved in emesis (see Figure 44.1), it is generally preferable to determine the cause of emetic episodes before using antiemetic drugs. Attention should always be directed towards treatment of the primary disease condition. In addition, fluid and electrolyte therapy, dietary management and proper nursing are fundamental to the rational use of antiemetic drugs.

Classification

Antiemetics represent a variety of classes, which are useful in different emetic conditions. Antiemetics may act peripherally either to reduce afferent input from receptors or inhibit efferent components of the reflex response. They may also act centrally by blocking relays to and through the chemoreceptor trigger zone and vomiting centre. Accordingly, antiemetics have been divided into two main classes, each consisting of several sub-classes.

I. Locally/Peripherally acting antiemetics

1. Demulcents and protectants
   e.g. kaolin, pectin and bismuth salts.
2. Gastric antacids and local anaesthetics
   e.g. magnesium hydroxide, aluminium hydroxide and benzocaine.
3. Anticholinergics
   e.g. glycopyrronium, methscopolamine and propantheline.
4. Prokinetics
   e.g. domperidone and cisapride.

II. Centrally acting antiemetics

1. H_1- receptor antagonists

e.g. meclozine, cyclizine, cinnarizine, dimenhydrinate and promethazine.

2. D_2- receptor antagonists

a. Phenothiazine derivatives

e.g. chlorpromazine, prochlorperazine and acepromazine.

b. Butyrophenones

e.g. droperidol and haloperidol.

c. Benzamides

e.g. metoclopramide, trimethobenzamide and alizapride.

3. 5-HT_3 receptor antagonists

e.g. ondansetron, granisetron, tropisetron, dolasetron, palonosetron and cyproheptadine.

4. Muscarinic receptor antagonists

e.g. hyoscine, dicycloverine and aminopentamide.

5. NK_1 receptor antagonist

e.g. aprepitant, maropitant and casopitant.

6. Miscellaneous drugs

a. Glucocorticoids

e.g. dexamethasone and methylprednisolone.

b. Benzodiazepines

e.g. diazepam, lorazepam and midazolam.

c. Cannabinoids

e.g. dronabinol and nabilone.

I. LOCALLY ACTING ANTIEMETICS

1. Demulcents and Protectants

Demulcents and protectants coat, protect, lubricate and sooth the irritated gastric mucous membranes, thereby suppressing the emetic stimuli originating from the gastric mucosa. These agents, however, have limited therapeutic use as antiemetics due to their unreliable action. Important examples of demulcents and protectants include kaolin, pectin and bismuth salts.

2. Gastric Antacids and Local Anaesthetics

Gastric antacids such as magnesium hydroxide and aluminium hydroxide are indicated when gastric acidity contributes to the underlying pathogenesis of vomiting. Topical anaesthetics such as benzocaine and tetracaine that desensitise gastric mucosa are occasionally included in oral preparations to control nausea and vomiting caused by GI irritation. However, their efficacy as antiemetics is not apparent.

Dose

Magnesium hydroxide or Aluminium hydroxide

Dogs & cats : 10 – 30 ml (total), PO, 3 times daily.

3. Anticholinergics

Some anticholinergic drugs which do not cross the blood-brain barrier well and thus have only peripheral action are used as antiemetics. These peripherally acting anticholinergics include glycopyrronium, propantheline, methscopolamine and isopropamide. Their ability to suppress emesis is probably related to inhibition of afferent vagal impulses, relief from gastrointestinal smooth muscle spasms and inhibition of gastro-enteric secretions. However, delayed gastric emptying caused by these agents may itself cause emesis; so anticholinergics should not be used for longer than 3 days in the vomiting animal.

Important side effects seen with peripherally acting anticholinergics include xerostomia, mydriasis, tachycardia, urinary retention, ileus and gastric retention. Of various anticholinergics, methscopolamine is not recommended in cats. Because of their anticholinergic properties, these drugs should not be used in combination with drugs whose actions are dependent on cholinergic activity in ganglia or smooth muscles e.g. metoclopramide, cisapride and opioids. The detailed pharmacology of peripherally acting anticholinergics is given in Chapter 14.

Dose

As antiemetic

Glycopyrronium

Dogs and cats : 0.01 – 0.02 mg/kg, IM or IV, 2 to 3 times daily.

Isopropamide iodide

Dogs : 0.1 – 1.2 mg/kg, PO, 2 times daily.

Cats : 0.07 mg (total), 4 to 6 times daily.

Methscopolamine

Dogs : 0.3 – 1 mg/kg, PO, 3 times daily.

Propantheline

Dogs : 0.25 mg/kg, PO, 2 to 3 times daily.

Cats : 7.5 mg (total), PO, at 1 to 3 days interval.

4. Prokinetics

Prokinetic agents are drugs which promote gastroduodenal peristalsis and accelerate gastric emptying. Delayed gastric emptying and/or gastric stasis most often results in gastric distension, retention of food and vomiting. Thus some prokinetic agents have an important role to play in the clinical management of vomiting.

Domperidone

Domperidone is an antidopaminergic drug that possesses both prokinetic and antiemetic properties. It stimulates gastric motility by inhibiting D_2 receptors in the stomach. Domperidone does not cross blood-brain barrier in sufficient concentrations, but it does act on the CTZ not protected by blood-brain barrier. Its antiemetic effect in thus primarily seems to be restricted to increasing gastric contractions. Accordingly, its antiemetic effect is lower than that of metoclopramide, which acts both centrally and peripherally (see for detail Chapter 43). Domperidone may be used either alone or together with metoclopramide, cyclizine and $5HT_3$ receptor antagonists (such as granisetron) in the treatment of nausea and vomiting. Domperidone can be administered orally, intravenously, intramuscularly or rectally for control of nausea and vomiting.

Dose

As antiemetic

Dogs : 0.1 – 0.5 mg/kg, IM

: 0.5 – 1 mg/kg, PO.

Cisapride

Cisapride, a benzamide, is structurally related to procainamide. Its prokinetic effect closely resembles that of metoclopramide and domperidone, but it is not very effective antiemetic drug. It has no antidopaminergic action and does not act on CTZ. It mediates its prokinetic effect through stimulating 5-HT_4 receptors in the stomach (See for detail Chapter 43).

Dose (As antiemetic)

Dogs : 0.1 – 0.5 mg/kg, PO, 3 times daily.

II. CENTRALLY ACTING ANTIEMETICS

Centrally acting antiemetics block impulses at higher centres and/or at emetic centre. They include many groups of drug, which may be considered to possess a limited or broad antiemetic effect.

1. H_1- Receptor Antagonists/Antihistamines

The antiemetic properties among the histamine H_1- receptor antagonists are restricted to drugs, which are piperazine (e.g. cyclizine and meclozine), ethanolamine (e.g diphenhydramine and dimenhydrinate) and phenothiazine (e.g. promethazine) derivatives. These drugs are useful mainly in motion sickness or inner ear disease that is mediated by the vestibular apparatus. These drugs have little or no activity against vomiting produced by substances acting directly on the CTZ. All anti-motion sickness drugs are more effective when taken 30-60 minutes before commencing journey. Once sickness has started, it is more difficult to control vomiting and for it high doses/parenteral administration may be required.

Meclozine

Meclozine (Meclizine) HCl is a first-generation antihistamine of the piperazine class. In addition to anti-histaminic properties, it possesses antiemetic, CNS depressant, antispasmodic and local anaesthetic activities. The exact mechanism of action for its antiemetic and anti-motion sickness effects are not completely understood, but it is thought that prevention of emesis is a result of the drug's central antihistaminic, anticholinergic and CNS depressant activities. The drug depresses labyrinth excitability and vestibular stimulation, and it may affect the medullary chemoreceptor trigger zone.

Important side effects of meclozine in therapeutic doses may include sedation, dry mouth and tachycardia. In some cases, CNS stimulation has been reported. Overdosage of meclozine may result in drowsiness alternating with hyper-excitability. Meclozine is principally used in small animals as an antiemetic and for treatment and prevention of motion sickness. In humans, it is used to treat symptoms of motion sickness and for management of vertigo that arises from diseases affecting the vestibular system. It is a relatively longer acting drug.

Dose

For motion sickness and emesis

Dogs : 2 – 6 mg/kg, PO. For motion sickness drug is given 1 hour before travelling.

Cats : 1 – 4 mg/kg, PO, once daily.

Cyclizine

Cyclizine HCl is a piperazine derivative with histamine H_1-receptor antagonist activity. Its action and pharmacological properties resemble those of meclozine. The precise mechanism of action in inhibiting the symptoms of motion sickness is not well understood, but similar to meclozine it is postulated to act directly on the labyrinthine apparatus and on the chemoreceptor trigger zone. Cyclizine also exerts a central anticholinergic action. Its action may last for 8-12 hours and the main side effect is drowsiness. Cyclizine has been recommended to treat nausea, vomiting and dizziness associated with motion sickness, vertigo and post-operatively following administration of general anaesthesia and opioids.

Dose

For motion sickness

Dogs : 25 – 100 mg (total), PO, in divided doses.

: 4 mg/kg, IM, 3 times daily.

Cinnarizine

Cinnarizine is a piperazine derivative with histamine H_1-receptor blocking and calcium channel blocking actions. It is an effective antivertigo drug and also provides protection against motion sickness. It probably acts by inhibiting influx of Ca^{++} from endolymph into the vestibular sensory cell, which mediates labyrinthine reflexes. It thus interferes with the signal transmission between vestibular apparatus of the inner ear and vomiting centre of the hypothalamus. Cinnarizine is also considered a nootropic drug because of its vaso-relaxating abilities in brain, which occurs due to calcium channel blockade. Important side effects of cinnarizine are drowsiness and blurred vision.

Dimenhydrinate

Dimenhydrinate is an ethanolamine derivative that has antihistaminic, antiemetic, anticholinergic, CNS depressant and local anaesthetic effects (see Table 27.2). However, it is used mainly for its anti-emetic/motion sickness effect. Its exact mechanism of action for this indication is unknown but is thought to inhibit vestibular stimulation. The anticholinergic actions of dimenhydrinate may play role in blocking acetylcholine stimulation of the vestibular and reticular systems. Antiemetic effect occurs almost immediately after IV injection and its duration of action is 3-6 hours.

Important side effects of dimenhydrinate are related to CNS depression (lethargy and somnolence) and anticholinergic effects (dry mouth and urinary retention). Gastrointestinal effects like diarrhoea, vomiting and anorexia are less common.

Dose

For prevention and treatment of motion sickness

Dogs : 8 mg/kg, PO, 3 times daily.

Cats : 12.5 mg (total), PO, 3 times daily.

Diphenhydramine

Diphenhydramine is an ethanolamine derivative antihistamine that also possesses sedative, anticholinergic, antitussive and antiemetic activities (see Table 27.2). In veterinary medicine, it is used mainly for its antihistaminic effects, but may also be used for the treatment and prevention of motion sickness and as antiemetic in dogs and cats. Its side effects/adverse effects are just like dimenhydrinate. The pharmacology of diphenhydramine is described in Chapter 27.

Dose

For motion sickness and emesis

Dogs & cats : 2 – 4 mg/kg, PO or IM, 3 to 4 times daily.

Promethazine

Promethazine is a first-generation H_1 receptor antagonist of the phenothiazine chemical class used mainly as an antiemetic. It also possesses considerable anticholinergic and sedative activities and is occasionally used as a hypnotic, particularly when benzodiazepines are contraindicated. Promethazine may also be used as an anti-allergic to treat allergic rhinitis and as anti-tussive, together with codeine or dextromethorphan, to treat cough

Previously it was used as an antipsychotic in human patients. Drowsiness, dry mouth, fatigue and constipation are some of the important side effects of promethazine.

Dose

For emesis

Dogs & cats : 2 mg/kg, PO or IM, once daily.

2. D_2- Receptors Antagonists/Antidopaminergic Drugs

A large number of agents from different chemical structural groups act as antagonist of dopamine D_2 receptor. Some of these agents are potent antiemetics. These agents block dopamine D_2 receptors in the CTZ and can antagonise apomorphine induced vomiting. Antidopaminergic drugs are effective against nausea and vomiting induced by several conditions including drugs, general anaesthesia, malignancy, cancer chemotherapy, radiation sickness and systemic diseases (e.g. gastroenteritis, uraemia and liver disease). However, they are not much useful in motion sickness, labyrinthitis and other vestibular pathways mediated vomiting probably because these pathways do not involve dopaminergic neurons. Many of these drugs are also useful as antipsychotics/neuroleptics; however, their antiemetic doses are much lower than the antipsychotic doses.

a. Phenothiazine Derivatives

Phenothiazine derivatives include broad-spectrum antiemetics which control emesis induced by most central causes other than labyrinthine stimulation. Selection of a particular phenothiazine may be based on availability and avoidance of adverse effects.

Chlorpromazine

Chlorpromazine HCl is a propylamino phenothiazine derivative that has sedative-tranquillizing/neuroleptic and antiemetic properties. The clinical use of chlorpromazine as a neuroleptic agent has diminished, but the drug is still used for its antiemetic effects in small animals and occasionally as a pre-anaesthetic and tranquillizer-sedative. As an antiemetic, it blocks dopamine receptors in the chemoreceptor trigger zone, possibly by interfering with regulation of dopamine permeability at synaptic membranes. At high doses, it appears to have direct inhibitory actions on the medullary vomiting centre. In addition to its antidopaminergic activity, chlorpromazine possesses antiadrenergic, antihistaminergic and weak anticholinergic activities.

As an antiemetic, chlorpromazine is more effective in dogs than in cats. Chlorpromazine can inhibit apomorphine and morphine induced vomiting in dogs, but not in cats. It has a biological half-life of about 6 hours in dogs. Important side effects of chlorpromazine include drowsiness, sedation and hypotension. Hypotension due to alpha-adrenoceptor blocking effect is a potentially serious side effect that occurs more frequently in dehydrated animals. Therefore, animals should be adequately hydrated before administering the drug. Besides sedation, other side effects are rarely seen in dogs, unless the drug is overdosed.

Dose

For emesis

Dogs & cats : 0.2 – 0.5 mg/kg, IM or SC, 3 to 4 times daily.
: 0.05 – 0.5 mg/kg, IV, 3 to 4 times daily
Dogs : 3.3 mg/kg, PO, 3 to 4 times daily.

Prochlorperazine

Prochlorperazine is a piperazine phenothiazine derivative with the basic pharmacology similar to that of other phenothiazines. It has strong antidopaminergic and antiemetic effects, weak anticholinergic effect and moderate sedative effect. It has selective antivertigo and antiemetic effects, which are probably due to its labyrinthine suppressant action. Following intramuscular injection the antiemetic action is evident within 5 to 10 minutes and lasts for 3 to 4 hours. After oral administration, the onset of action is slow but the duration is long (approximately 6 hours).

Prochlorperazine is most often used for the short-time treatment of nausea and vomiting and vertigo. It is also useful for nausea and vomiting caused by chemotherapy, radiation therapy and in the pre- and post-operative conditions. Important side effects of prochlorperazine include sedation and hypotension. Hypersensitivities to prochlorperazine can occur and there is cross-reactivity with other drugs in the phenothiazine class. Prochlorperazine is available in different dosage forms including oral liquid, tablet, injection and suppositories. Administration by injection is considered more effective in vertigo associated with vomiting.

Dose

Dogs : 0.25 – 0.5 mg/kg, IM, 2 to 3 times daily
: 1 mg/kg, PO, 2 times daily.
Cats : 0.13 mg/kg, IM, 2 times daily.
: 0.5 mg/kg, PO, mixed in food.

Acepromazine

Acepromazine is a phenothiazine derivative tranquillizer-sedative with additional antiemetic, antispasmodic and hypothermic activities. It blocks post-synaptic dopamine receptors in the CNS and may also inhibit the release of dopamine. In addition, acepromazine like other phenothiazines possesses varying degrees of anticholinergic, antihistaminic and alpha-adrenergic blocking actions. The detailed pharmacology of acepromazine has been given in Chapter 21.

Dose

For emesis

Dogs & cats : 0.06 – 0.25 mg/kg, IM or SC.
: 1 – 3 mg/kg, PO.

Other phenothiazine derivatives

Several other phenothiazine derivatives used as antiemetic include promazine, perphenazine and thiethylperazine.

Dose

Promazine
Dogs and cats : 0.13 mg/kg, IM, 3 times daily.
Perphenazine
Dogs & cats : 0.04 mg/kg, IM, 4 times daily.
Thiethylperazine
Dogs & cats : 0.13 – 0.44 mg/kg, IM, 3 times daily.

b. Butyrophenone Derivatives

Butyrophenones such as droperidol and haloperidol act as competitive antagonists of dopamine at D_2 receptors. These drugs are used primarily as antipsychotic drugs in human medicine, but have also antiemetic effects similar to those of phenothiazine derivatives. Although generally considered to be only moderately

effective, butyrophenones in frequent and high doses are nearly as effective as high dose metoclopramide in preventing emesis due to antineoplastic drugs. These drugs, however, are less used as antiemetics in veterinary medicine because of their adverse effects in animals. They have pharmacological activity similar to piperazine phenothiazines and show extrapyramidal side effects.

Dose

Droperidol

Dogs : 0.02 – 0.1 mg/kg, PO or IM, every 2 to 4 days.

Haloperidol

Dogs : 0.02 – 0.1 mg/kg, PO or IM, every 4 days.

c. Benzamides

Substituted benzamides like metoclopramide and trimethobenzamide are good D_2 receptor blockers and potent antiemetic.

Metoclopramide

Metoclopramide is a substituted benzamide that is therapeutically used as gastric prokinetic and anti-emetic drug. Metoclopramide inhibits vomiting associated with activation of central dopaminergic D_2 receptors in the CTZ. In high doses, it also inhibits 5-HT_3 receptors on CTZ. Metoclopramide may also act peripherally to diminish the severity of vomiting through its effects on motility by preventing gastric stasis and retrograde peristalsis that precedes vomiting (see for detail Prokinetics, Chapter 43). The central antiemetic effect of metoclopramide is likely to be superior to its peripheral prokinetic effect.

Metoclopramide effectively antagonises apomorphine induced vomiting and is about 20 times as potent as phenothiazines. It is indicated for control of emesis induced by wide-variety of blood-borne and peripheral causes. The main indications of metoclopramide include severe and intractable emesis due to chemotherapy as well as nausea and vomiting associated with delayed gastric emptying, gastroesophageal reflux, reflux gastritis, peptic ulcerations, infections etc. Main side effects of metoclopramide include nervousness, restlessness, listlessness, GI disorders and depression. The pharmacology of metoclopramide has been given in chapter 43.

Dose

For emesis

Dogs & cats : 0.1–0.3 mg/kg, PO, SC, IM, or IV, 3 times daily.

: 0.02 mg/kg/hour continuous IV infusion.

: 1 – 2 mg/kg, IV infusion every 24 hours for severe emesis.

Trimethobenzamide

Trimethobenzamide is a benzamide antiemetic used to prevent nausea and vomiting. The specific mechanism through which trimethobenzamide acts is unknown, but it is believed to mediate its effect through antagonism of dopamine receptor in the chemoreceptor trigger zone (CTZ). Trimethobenzamide is generally considered a potent antiemetic with no/less effects on the serotonergic or histaminergic systems. However, its efficacy is less than that of metoclopramide. Side effects are not common but drowsiness, dizziness, extrapyramidal symptoms and convulsion have been reported in human beings. Trimethobenzamide seems to be effective for the control of vomiting caused by drug therapy, toxins (uraemia) and metabolic disturbances.

Dose

Dogs : 3 mg/kg, IM, 3 times daily.

Alizapride

Alizapride is a new substituted benzamide with prokinetic and antiemetic effects. It is structurally and pharmacologically similar to metoclopramide. Alizapride is well absorbed from the gastrointestinal tract and is mainly excreted unchanged in the urine. It is used in the treatment of nausea and vomiting, including postoperative nausea and vomiting.

Pimozide

Pimozide is an antipsychotic drug of the diphenyl-butylpiperidine class. It has shown particularly long duration of antiemetic action (up to 6 days). Pimozide has no significant sedative properties, but can produce extrapyramidal side effects, including tardive dyskinesia. Pimozide is occasionally used in dogs against drug-induced emesis.

3. 5-HT_3-Receptor Antagonists

Serotonin 5-HT_3 receptor antagonists are a relatively new group of anti-emetic drugs, used mainly to treat chemotherapy induced nausea and vomiting. Serotonin antagonists used for antiemetic therapy include ondansetron, granisetron, tropistron, dolasetron, azasetron, palonosetron and cyproheptadine. These drugs share their mechanism of action, but may have different chemical structures and exhibit differences in affinity for the receptor, dose response and duration of effect.

Ondansetron

Ondansetron HCl is a selective serotonin 5-HT_3 receptor antagonist developed in 1984. It is considered the prototype drug of its class and has been used in human and animals to control emesis induced by asplastin (a potent emetogen), chemotherapeutic agents and radiation treatment.

Mechanism of action and pharmacological effects

Ondansetron and related drugs act selectively at the 5-HT_3 receptor, a subtype of serotonin receptor found in terminals of the vagus nerve and in certain areas of brain. As discussed earlier, serotonin released by the enterochromaffin cells of the small intestine in response to chemotherapeutic agents stimulates vagal afferents (via 5-HT_3 receptors) and initiates vomiting reflex. The high concentration of serotonin receptors present in the central STN and chemoreceptor trigger zone (CTZ) areas are also involved in nausea and vomiting. The 5-HT_3 receptor antagonists suppress vomiting and nausea by inhibiting serotonin binding to the 5-HT_3 receptors at both peripheral and central sites. Antagonising the peripheral receptors may also cause a prokinetic effect and thus may help to control nausea. It has little effect on vomiting caused by motion sickness and does not have any effect on dopamine and muscarinic receptors.

Pharmacokinetics

The pharmacokinetics of ondansetron in veterinary species has not been well studied. Ondansetron is well absorbed after oral administration. It is metabolized mainly in the liver by the cytochrome P-450 enzyme system.

Side/Adverse effects

Although clinical experience with the drug is limited in veterinary practice, experimental studies suggest that ondansetron is very safe showing minimal toxicity at doses much higher than those needed to abolish vomiting. Since ondansetron is not an

antagonist of dopamine receptor, it does not cause extrapyramidal side effects associated with metoclopramide.

Contraindication and drug interactions

No important contraindications of ondansetron are identified in animals. Similarly no significant drug interactions are reported for animals. The clinical effects of ondansetron and related drugs can be potentiated by combining them with dexamethasone.

Clinical uses

Ondansetron has been used in small animals suffering from refractory vomiting that has not responded to other antiemetics i.e. vomiting induced by chemotherapy, Parvovirus and hepatic lipidosis. However, nausea and emesis via gastrointestinal irritation respond less to ondansetron. In particular, ondansetron has been used successfully in puppies with Parvovirus enteritis that vomit profusely despite therapy with chlorpromazine or metoclopramide. Ondansetron does not block dopamine receptors and apomorphine or motion sickness induced vomiting. Ondansetron and other 5-HT_3 antagonists are mostly administered prior to chemotherapy for getting full efficacy.

Dose

Dogs : 0.1 – 0.2 mg/kg, SC, 3 times daily.
: 0.1 – 0.5 mg/kg, IV, up to 4 times daily.
: 0.5 mg/kg/hour, IV infusion.

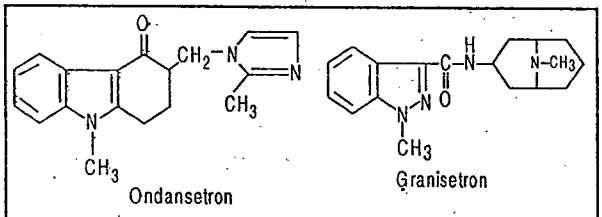


Fig. 44.3. Chemical structures of selected serotonin 5-HT_3 receptor antagonist anti-emetics.

Granisetron

Granisetron is another 5-HT_3 antagonist available for oral or IV administration to minimise chemotherapy-induced emesis. It is about 10 times more potent than ondansetron and is considered more effective during the repeat cycle of chemotherapy to prevent nausea and vomiting. Significant side/adverse effects, contraindications and drug interactions have not been reported for the granisetron in animals. There has been only limited use of this drug in veterinary practice.

Dose

Dogs and cats : 0.01 mg/kg, IV.

Cyproheptadine

Cyproheptadine is a non-selective serotonin antagonist with anticholinergic and antihistaminergic effects. Although its use as an appetite stimulant has been documented, the clinical efficacy of cyproheptadine as an antiemetic in small animals has been less well established.

Dose

Dogs : 1.1 mg/kg, PO, 2 to 3 times daily.
Cats : 2 – 4 mg (total), PO, 1 to 2 times daily.

4. Muscarinic Receptor Antagonists/Anticholinergics

Selected antimuscarinic drugs which penetrate into CNS are occasionally used to control motion sickness. The belladonna alkaloids, especially hyoscine (scopolamine) and synthetic compounds such as dicycloverine hydrochloride and isopropamide iodide are effective antiemetics. Antimuscarinic drugs like hyoscine are effective against nausea and vomiting of labyrinthine origin but are ineffective against substances which directly act on the CTZ. These drugs are not generally used in cats, probably because of potential adverse effects.

Hyoscine

Hyoscine (Scopolamine) is a naturally occurring alkaloid that competitively blocks action of acetylcholine on the muscarinic receptors (see for detail Chapter 14). It effectively blocks cholinergic afferent impulses to the vomiting centre and decreases conduction at labyrinthine receptors and vestibular pathways. Hyoscine is considered as the most effective drug against motion sickness, but is not effective in vomiting of other aetiologies. Hyoscine has brief duration of action in animals, which limits its usefulness in motion sickness. It produces anticholinergic effects and sedation in animals, except in cat in which it produces excitement. The pharmacology of hyoscine is given in Chapter 14.

Dose

For motion sickness and emesis

Dogs : 0.03 mg/kg, 4 times daily.

Dicycloverine

Dicycloverine (Dicyclomine) is a tertiary amine atropine substitute that has antimuscarinic activity like hyoscine. In addition to antispasmodic action (principal action), it has antiemetic property. Dicycloverine is used clinically to treat intestinal hypermotility and symptoms of Irritable Bowel Syndrome (see Chapter 14). It is also used in motion sickness to prevent nausea and vomiting. Dicycloverine can cause a range of anticholinergic side effects such as dry mouth, nausea and at higher doses euphorient and hallucinogenic effects.

Dose

For motion sickness and emesis

Dogs : 5 – 10 mg (total), PO, 3 times daily.

Aminopentamide

Aminopentamide is an antimuscarinic drug that may have some central-acting activity. It is moderately effective in vomiting animals, but seldom works as effective as chlorpromazine or metoclopramide.

5. NK_1-Receptor Antagonists

Neurokinin 1 (NK_1) receptor antagonists are a novel class of drugs which possess unique antidepressant, anxiolytic and antiemetic properties. As discussed earlier (Chapter 28), the NK_1-receptor belongs to the family of tachykinin receptors and are found in both the central and peripheral nervous systems. The binding of substance P (SP) to the NK_1 receptor has been associated with pain, stress, emesis, contraction of smooth muscles and inflammation. NK_1 receptor antagonists have, therefore, found importance in migraine, emesis and some psychiatric disorders. The main clinical use for which these drugs have been applied so far, however, is as antiemetics in both human and veterinary medicines.

Aprepitant

Aprepitant is a recently introduced anti-emetic drug. It mediates its effect by selectively blocking the neurokinin 1 (NK_1)

receptor located in the central nervous system and the peripheral nervous system. The NK_1 receptor is found in high concentrations in the vomiting centre of the brain and when activated it results in a vomiting. In human beings, it is moderately to well absorbed to the extent of 60-65%. Metabolism occurs mainly through hepatic cytochrome P-450 system. Seven metabolites of aprepitant, which are only weakly active, have been identified in human plasma.

Aprepitant is recommended mainly in human medicine for prevention of acute and delayed chemotherapy-induced nausea and vomiting and for prevention of postoperative nausea and vomiting. Aprepitant has also been shown to increase the activity of the 5-HT_3 receptor antagonists (e.g. ondansetron) and the corticosteroids (e.g. dexamethasone), which are also used to prevent chemotherapy-induced nausea and vomiting. Aprepitant may also be considered useful in the treatment of cyclic vomiting syndrome. Important side/adverse effects associated with aprepitant in humans include weakness, tiredness, dizziness, stomach pain, loss of appetite, etc. It is taken orally in the form of a capsule.

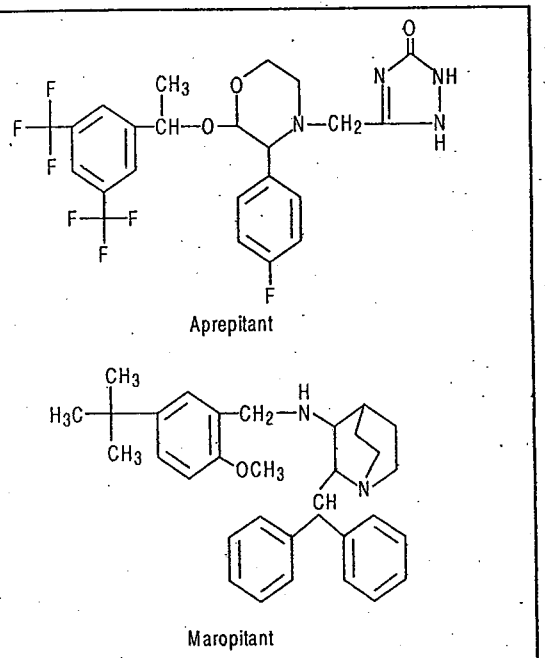


Fig. 44.4. Chemical structures of selected NK_1-receptor antagonist anti-emetics.

Maropitant

Maropitant is a neurokinin 1 (NK_1) receptor antagonist, developed specifically for the motion sickness and vomiting in dogs. Similar to aprepitant, it blocks neurokinin 1 (NK_1) receptor in the central nervous system (CNS) and prevents emetogenic action of substance P on the vomiting centre. By inhibiting the binding of substance P within the emetic centre, maropitant provides broad-spectrum effectiveness against neural (central) and humoral (peripheral) causes of vomiting. *In vivo* model studies in dogs have shown that maropitant has antiemetic effectiveness against both central and peripheral emetogens including apomorphine and syrup of ipecacuanha.

Maropitant is moderately absorbed (24%) after oral administration in dogs, but after SC injection its bioavailability increases to about 90%. The lesser oral bioavailability of maropitant has been related mainly to higher first-pass metabolism. Maropitant is largely metabolised in liver by the cytochrome P-450 system mainly involving CYP2D15 and CYP3A12 isozymes. Maropitant is well tolerated in dogs, but some animals display signs of salivation, loss of appetite, diarrhoea and bloody stool. Oral tablets in the salt form of maropitant citrate are available for the prevention of acute vomiting and motion sickness vomiting in dogs. Tablets are generally administered 2 hours before the travel.

Dose

For prevention of acute vomiting

Dogs : 2 mg/kg, PO, once daily for up to 5 consecutive days.

For prevention of vomiting due to motion sickness

Dogs : 8 mg/kg, PO, once daily for up to 2 days.

Other drugs

Other NK1 receptor antagonists used as anti-emetic agents include fosaprepitant, casopitant and vestipitant. Fosaprepitant is a pro-drug of aprepitant and is used intravenously. casopitant and vestipitant are currently undergoing clinical trials for the treatment of chemotherapy-induced nausea and vomiting.

6. Miscellaneous Drugs

Several miscellaneous drugs act as potent antiemetics. These drugs may be used either alone or in conjunction with other agents for prevention and/or treatment of various types of emesis.

a. Glucocorticoids

Glucocorticoids, particularly dexamethasone, possess antiemetic effects, although the antiemetic mechanism is not clearly understood. Dexamethasone and methylprednisolone have been used in human beings to control vomiting associated with chemotherapy. Glucocorticoids also appear to act in additive or synergistic fashion when combined with other antiemetics like metoclopramide and ondansetron for highly emetogenic regimen. They also serve to reduce certain side effects of primary antiemetics. Although glucocorticoids might prove beneficial for control of vomiting, their side effects, particularly their metabolic effects should lead to cautious use (see for detail Chapter 51).

b. Benzodiazepines

Benzodiazepines have weak antiemetic property, which is primarily derived from their sedative and anxiolytic properties. These drugs are used mainly as adjunct to metoclopramide or ondansetron and are recommended to control psychogenic or behavioural vomiting. Diazepam used in cats produce anti-emetic action. Other benzodiazepines which can be used as an adjunct treatment for nausea and vomiting include midazolam and lorazepam.

Dose

Diazepam

Cats : 0.05 – 0.4 mg/kg, IV, to be repeated after 24 hours.

: 1 – 2 mg (total), PO, to be repeated after 24 hours.

c. Cannabinoids

Dronabinol

Tetrahydrocannabinol (THC), also known as delta-9 tetrahydrocannabinol (Δ^9 THC), is the active principle of the hallucinogen of the cannabis plant (*Cannabis indica and Cannabis sativa*). THC possesses mild to moderate analgesic

Table 44.1: Clinical use of some centrally acting antiemetics in different types of emesis.

Antiemetic	Types of emesis
1. H_1- receptor antagonists	
Cyclizine	Motion sickness.
Meclozine	Motion sickness.
Cinnarizine	Motion sickness and vestibular disturbance.
2. D_2-Receptor antagonists	
Phenothiazines	Vomiting caused by uraemia, radiation and viral enteritis (drug of choice).
Metoclopramide	Vomiting caused by uraemia, radiation, gastrointestinal disorders and cytotoxic drugs.
3. 5-HT_3-Receptor antagonists	
Ondansetron	Vomiting caused by cytotoxic antineoplastic drugs (drug of choice), radiations and surgery (post-operative vomiting).
4. Muscarinic receptor antagonists	
Hyoscine	Motion sickness (drug of choice).
5. NK_1-receptor antagonists	
Aprepitant	Nausea and vomiting caused by cytotoxic antineoplastic drugs.
Maropitant	Prevention of motion sickness.

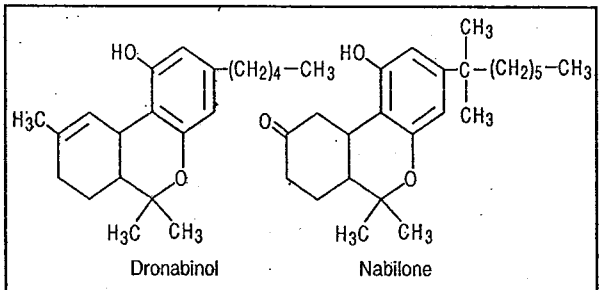


Fig. 44.5. Chemical structures of selected cannabinoid anti-emetics

effect, psychoactive effect, appetite stimulant effect and anti-emetic effect. The biological actions of THC result from its binding to the cannabinoid receptor CB_1, located mainly in the central nervous system, and the CB_2 receptor, mainly present in cells of the immune system.

Dronabinol, the chemically synthesized and a pure isomer of THC, is available commercially in several countries as an appetite stimulant and an anti-emetic agent against moderately emetogenic chemotherapy. It probably acts at a higher centre or at vomiting centre itself. However, disorientation and other central effects of Δ^9 THC limit its clinical usefulness. Concurrent use of prochlorperazine in low doses can reduce the incidence of dysphoria that accompanies cannabinoid administration.

Nabilone

Nabilone is a synthetic cannabinoid and an analogue of dronabinol. It has been recommended for as an antiemetic and an adjunct analgesic for neuropathic pain. As an anti-emetic, it is effective against agents those induce vomiting due to stimulation of CTZ. It is comparatively less hallucinogenic and more antiemetic than dronabinol. Nabilone has been used for the chemotherapy induced vomiting in human beings for patients who cannot tolerate other antiemetic drugs or are unresponsive to them.

DRUGS AFFECTING APPETITE

The control of appetite and its pharmacological modulation is complex and incompletely understood. Appetite is regulated by hunger and satiety centres in the hypothalamic nuclei and other components of the limbic system. Several major neurotransmitters including norepinephrine (through α_2-adrenoceptor), dopamine (possibly through D_1 receptor), serotonin, and possibly γ-aminobutyric acid are involved in the control of appetite. Several neuropeptides have also been implicated in the control of appetite because of their ability to modulate neurotransmitter release. Endocannabinoids, endogenous opioid peptides and pancreatic polypeptides are associated with increased appetite, whereas calcitonin, cholecystokinin and corticotropin releasing factor inhibit appetite. The neurons involved are responsive to glucose concentration, amino acids levels, fat stores, etc. Several internal and external influences can also alter appetite and feeding behaviour of the animals. Factors related to feed or food (palatability), physiological conditions (e.g. oestrus and pregnancy) and nutritional status of animal may also alter feeding response and appetite.

Disorders of appetite are common in animals. Anorexia or inappetance accompanies diseases and external stressful conditions. Overeating associated with obesity also occurs fairly common in animals. Drugs are available which modify appetite, but disorders affecting appetite are best approached by treating the underlying disease process, regulating diet and good nursing care of the sick animal.

APPETITE STIMULANTS

Appetite stimulants, also called orexigenics or appetisers, are a diverse group of drugs used to enhance appetite in patients suffering from debilitating diseases such as cancer. These drugs are also used to prevent undesired weight loss in geriatric individuals and in patients suffering from wasting diseases. In veterinary practice, several drugs have been used to promote appetite in animals, although no definitive evidence is available to prove their worth. Drugs used as appetite stimulants in monogastric animals include benzodiazepines, cyproheptadine, glucocorticoids, anabolic steroids and B vitamins. The appetite stimulants are used mainly for the short-term stimulation of appetite.

1. Benzodiazepines

Benzodiazepines such as diazepam and oxazepam have been used for short-term stimulation of appetite, most frequently in cats and less frequently in horses, dogs and goats. Benzodiazepines induce appetite probably through GABA mediated pathways and central inhibition of the satiety centre in hypothalamus, and/or alleviation of anxiety (see also Chapter 23). The appetite stimulant effect of benzodiazepines usually varies with route of administration and may diminish after 2 to 3 treatment.

Diazepam is a more effective appetite stimulant, but it also has a greater sedative effect than oxazepam. Diazepam and oxazepam produce sedation, non-purposeful eating, hepatic failure and behaviour changes in cats and dogs, and sedation, muscle fasciculation and weakness in horses. Diazepam is administered both orally and parenterally, while oxazepam is given only orally. The detailed pharmacology of diazepam and oxazepam has been discussed in Chapters 21 and 23.

Dose

Diazepam

Cats	:	1 – 2 mg (total), PO, once daily.
Dogs & cats	:	0.05 – 0.15 mg/kg, IV, 1 to 3 times daily.
Horses	:	0.02 mg/kg, IV, 2 to 3 times in a 24-48 hour period.
Goats	:	0.04 mg/kg, IV.

Oxazepam

Cats	:	2 mg (total), PO, 1 to 2 times daily.
Dogs	:	0.3 – 0.4 mg/kg, PO, 1 to 3 times daily.

Note: After IV administration of benzodiazepines, eating may begin within a few seconds, so food should be offered to the animal immediately after dosing.

2. Cyproheptadine

Cyproheptadine is an antihistamine (H_1 blocker) with antiserotonin action. It promotes appetite by inhibition of serotonergic receptors, which control satiety centre. Inhibition of satiety centre in the hypothalamus promotes appetite, weight gain and growth. Cyproheptadine is used clinically in cats and humans, but is less frequently employed in dogs. The pharmacological effects of cyproheptadine develop slowly and may take 24 hours for desirable effect.

Important side/adverse effects of cyproheptadine in some cats (about 20%) include CNS excitement, aggregation, vomiting and anticholinergic effects. High doses in dogs may produce polyphagia.

Dose

Cats	:	2 – 4 mg (total), PO, 1 to 2 times daily for 1 week.
Dogs	:	5 – 20 mg (total) PO, 1 to 2 times daily.

3. Glucocorticoids

Glucocorticoids like prednisone, prednisolone and dexamethasone are used occasionally in dogs and other species to enhance feed intake. Although the precise mechanism by which glucocorticoids stimulate appetite in not known, steroids induced euphoria probably results in increased feed-intake. Glucocorticoids also increase gluconeogenesis and antagonise insulin for an overall hyperglycaemic effect. Continued use of glucocorticoids may result in decreased gastric mucus production and several other adverse effects as mentioned in Chapter 51.

Dose

Prednisolone

Dogs	:	0.25 – 0.5 mg/kg, PO, once daily or every alternate day.

4. Anabolic Steroids

Anabolic steroids are synthetic analogues of testosterone. They have high protein-anabolic activity but possess minimal androgenic effects (see for detail Chapter 52). Anabolic steroids promote appetite, weight gain and improved nitrogen balance, so they are used to reverse debilitation associated with surgery, trauma, illness, aging and glucocorticoid induced catabolism. As an appetite stimulant, stanozolol and boldenone undecylenate are approved for use in veterinary species, mainly in dogs and cats, and occasionally in horses. Anabolic steroids usually have delayed onset of action.

Important side/adverse effects of anabolic steroids seen after excessive dose or prolonged uses are hepatotoxicity, masculinisation, early closure of epiphyseal plates, infertility and occasional oedema formation. The pharmacology, side/adverse effects and clinical uses anabolic steroids have been given in Chapter 52.

Dose

Stanozolol

Dogs & cats	:	0.25 – 3 mg/kg, PO, once daily.
	:	2 – 10 mg/kg, IM, once weekly.

Boldenone undecylenate

Dogs & cats	:	5 mg (total), IM or SC, once daily.

5. Progestogens

Some synthetic progestogens like megestrol acetate possess growth promoting and appetite stimulant actions in humans with cancer and cachexia and may have similar effects in anorectic cats and dogs. In cats, megestrol acetate can induce a profound adrenocortical suppression, adrenal atrophy and diabetes mellitus, which may or may not be reversible (see also Chapter 52).

Dose

Megestrol acetate

Dogs	:	5 mg/kg, PO, once daily.

6. Miscellaneous Drugs

A large number of pharmaceuticals, nutrients and diet supplements possess appetite stimulant action and are occasionally used in animals. Mirtazapine, a tetracyclic antidepressant, and dronabinol, a synthetic cannabinoid, possess appetite stimulant action and are recommended in human medicine. Vitamin B preparations have been used orally and parenterally in debilitated animals, especially horses to promote appetite. Bitters such as nux vomica, quassia and gentian have been used as appetite stimulants in large animals because of their sialogogue and stomachic effects, but often with doubtful results. Fish oil (eicosapentaenoic acid or EPA) has been recommended as an alternative or complementary treatment for undesired weight loss. Alcoholic drinks and spices also function as appetisers due to their bitter taste and mild irritant action.

Whenever appetite stimulants are used in animals, it is important to quantitate food consumption to determine the effectiveness of such agents before continuing their

administration. If the patient is not eating adequately after 48 hours with appetite stimulant, other methods of nutritional support must be considered. In addition, force-feeding using a syringe or tube feeding may be considered.

APPETITE SUPPRESSANTS

Appetite suppressants (Anorexiants, anorectic agents, anorexigenic agents) are used in therapeutics as adjuncts in the management of obesity due to excessive food intake. These agents in general elevate the mood of patients and make them tolerant to dietary restrictions. These agents mainly stimulate the satiety centre in medial hypothalamus and block impulses reaching the eating centre in lateral hypothalamus. In addition, they increase plasma free fatty acid levels, which depress the eating centre.

Appetite suppressants or anorectic agents are not used in veterinary medicine to treat obesity because it is usually simpler to reduce the caloric intake of an animal. This may be achieved more effectively by feeding a nutritionally complete, but low calorie (~60-70% of that required for maintenance) and high fibre diet, and by reducing the quantity of diet. Further appetite suppressant drugs like amfetamine are not much effective in species like dog.

CARMINATIVES AND ANTI-FERMENTIVE DRUGS

CARMINATIVES

Carminatives are drugs or herbal preparations which promote expulsion of gases from the gastrointestinal tract and relieve the feeling of discomfort and distension of stomach. Acute distension of stomach or rumen can result in compromise of respiratory and cardiovascular functions in addition to epigastric pain. Carminatives are useful also in mild cases of simple bloat where they produce mild irritation of GI mucosa and relaxation of oesophageal sphincter for a short period, which helps in eructation of entrapped gases.

Many therapeutic agents have carminative action (e.g. volatile inhalant anaesthetics and alcohol), but only a few are used therapeutically for this purpose. Substances or preparations which are used for carminative effect contain usually volatile compounds and are mainly condiments and spices from plant source. The carminative action of these drugs, however, is not constant and their use for this specific purpose is declining, particularly in small animals. Some selected carminative compounds and their source(s), preparations and doses are given in Table 44.2.

Table 44.2: Selected carminatives with their source, preparations and doses in animals.

Compound	Source	Preparation	Dose (PO)	
			Dogs	Horses & cattle
1. Anise	Dried ripe seeds of *Pimpinella anisum.*	Powder	0.6 – 2 g	30 – 60 g
		Oil	0.03 – 0.2 ml	1 – 2.6 ml
2. Asafoetida	Oleo-gum resin collected from rhizome and root of *Ferula foetida* and *Ferula assafoetida.*	Powder	0.2 – 0.9 g	15 – 30 g
		Tincture	0.6 – 1.3 ml	15 – 60 ml
3. Camphor	A solid volatile oil (stearotene) obtained synthetically from wood of *Cinnamomum camphora.*	Powder	0.12 – 0.6 g	4 – 12 g
		Camphor water	2 – 4 ml	–
4. Caraway	Ripe dried fruits of *Carum carvi.*	Powder	0.6 – 2 g	30 – 60 g
		Oil	0.03 – 0.2 ml	1 – 2.6 ml
5. Cardamom	Dried ripe seeds of *Elettaria cardamomum.*	Powder	0.6 – 2 g	30 – 60 g
		Co. Tincture	1.3 – 4 ml	10 – 30 ml
6. Cinnamon	Bark and shoots of *Cinnamomum verum.*	Powder	0.6 – 1 g	8 – 15 g
		Oil	0.03 – 0.2 ml	1 – 2.5 ml
7. Clove	Dried flower heads of *Eugenia caryophyllus.*	Powder	0.3 – 0.6 g	H: 4 – 8 g C: 8 – 15 g
		Oil	0.03 – 0.2 ml	1 – 2.6 ml
8. Coriander	Dried ripe fruits of *Coriandrum sativum.*	Powder	0.6 – 2 g	30 – 60 g
		Oil	0.03 – 0.2 ml	–
9. Dill	Dried fruits of *Anethum graveolens.*	Powder	0.6 – 2 g	30 – 60 g
		Oil	0.03 – 0.2 ml	1 – 2.5 ml
10. Ginger	Rhizome of *Zingiber officinale.*	Powder	0.6 – 1.3 g	H: 15 – 30 g C: 30 – 60 g
11. Menthol	Solid volatile oil (strearotene) obtained from oil of peppermint. Prepared also synthetically.	Powder	0.03 – 0.12 g	0.3 – 1 g
12. Peppermint	Dried leaves and flowering tops of *Mentha piperita.*	Oil	0.03 – 0.2 ml	1.3 – 4 ml
		Spirit	0.12 – 0.6 ml	–
13. Spearmint (Mint)	Dried leaf and flowering tops of *Mentha spicata* or *Mentha cardiaca.*	Concentrated water	0.12 - 0.6 ml	–

Abbreviations: C = cattle; H = horses.

ANTI-FERMENTIVE AND ANTIFOAMING AGENTS

The normal process of fermentation of cellulose in the rumen results in production of gases, which are periodically eructed. Excessive production and accumulation of gas in rumen either in free form or in stable foam form often result in ruminal tympany or bloat. In majority of cases, the free gas bloat is secondary to other diseases such as oesophagitis, oesophageal obstruction, ruminitis and ruminal acidosis, and requires administration of antifermentive drugs along with ruminal intubation or trocharisation to allow release of gases. In frothy bloat, the gases of fermentation are trapped in stable foam and are difficult to remove by eructation or trocharisation. In such cases, the therapeutic approach requires administration of antifoaming agents to reduce foam stability and to promote release of free gases. Antifermentive agents are also used to suppress further fermentation in the rumen.

I. Antifermentive Agents

Antifermentive (Antizymotic) agents are drugs which prevent or decrease fermentation of gases in the stomach or rumen. These drugs are mostly given by stomach tube or as a drench. If a trochar and cannula are used, they may be injected directly into the rumen through the cannula.

Turpentine oil

Turpentine oil (T.T. oil) is a colourless volatile oil having a characteristic odour and taste, both of which become stronger and more disagreeable as the oil ages or is exposed to air. It is distilled from the crude turpentine, an oleo-resin, obtained from various species of pines (e.g. *Pinus palustris*). Turpentine oil is miscible with alcohol, ether and oils. Rectified turpentine oil is mainly used for internal use.

Pharmacological effects

Internally turpentine oil acts as a reflex sialogogue, carminative, antizymotic and antifoaming agent.

Side effects/Adverse effects

Turpentine oil is an irritant, so it should always be mixed with a demulcent. Systemic absorption of turpentine oil in high doses may lead to clinical signs of renal, CNS and GI toxicity including colic, diarrhoea, incoordination and excitement followed by coma. Turpentine oil should be used with care in lactating animals and food animals as it may taint milk and meat for up to 4 to 5 days.

Clinical uses

Turpentine oil is mainly used internally in the treatment of free gas bloat or frothy bloat in combination with linseed oil. Turpentine oil should not be mixed with fish oil as later stabilises the foam. Topically it has been used for abrasions and wounds and as a treatment for ectoparasites such as lice. When mixed with animal fat it has also been used as a chest rub or inhaler for nasal and throat ailments.

Dose

For tympany or free gas bloat

Sheep : 4 – 8 ml in 60 – 300 ml of raw linseed oil, PO or intra-ruminally.

Cattle : 15 – 30 ml in 300 – 600 ml of raw linseed oil, PO or intra-ruminally.

Formalin

Formalin is a 40% (37-41%) solution of formaldehyde in water. It is a colourless liquid with pungent odour and burning or slightly acidic taste. Internally, formalin is used as an antizymotic and intestinal antiseptic. Formalin solution is caustic, so for internal administration it should be freely diluted in water or saline solution and preferentially administered by stomach tube.

Dose

Cattle : 4 ml in 300 ml of water, PO, 3 times daily.

Sheep : 0.6 – 1 ml in 100 ml of water, PO, 3 times daily.

Other agents

Several other agents have shown good antifermentive properties but are less used for this purpose. These include phenol, cresol, lysol, chloral hydrate and oral antibiotics (e.g. penicillin).

II. Antifoaming Agents

Antifoaming or antifrothing agents are usually surfactants, which decrease the stability of foam in rumen and promote release of free gases. Antifoaming agents are used primarily in frothy bloat that requires urgent treatment.

1. Organic Silicones

Organic silicones are organic compounds containing silicon. They are polymers composed of long chains of alternating oxygen and silicon atoms with organic groups (e.g. methyl and phenyl) attached to the silicon. By altering the organic group and the degree of polymerization, many products are obtained, which are used for different purposes like adhesives, lubricants, synthetic rubber and prostheses.

Polydimethylsiloxane/Dimeticone

Polydimethylsiloxane (Polymerised methyl silicone, dimethyl polysilicone, PDMS), commonly called dimeticone (dimethicone), is an organic silicone that possesses methyl groups. It is widely used in veterinary practice for the treatment of frothy bloat. It exists as a viscous translucent liquid that is non-volatile, chemically inert, tasteless and non-toxic.

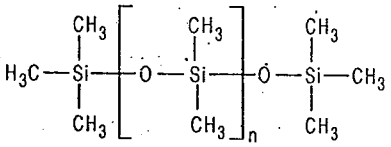


Fig. 44.6. Chemical structure of polydimethylsiloxane (Dimeticone).

Pharmacological effects

Polydimethylsiloxane decreases foam stability in frothy bloat by reducing the surface tension of gas bubbles causing them to coalesce. Polydimethylsiloxane does not reduce or prevent formation of gas in the digestive tract, rather it increases the rate at which it exits the body. It is not absorbed from the GI tract.

Side effects/Adverse effects

Polydimethylsiloxane in normal doses is non-toxic as it largely remains unabsorbed in GI tract. In higher doses it may cause constipation or diarrhoea.

Clinical uses

Polydimethylsiloxane is used medically in the treatment of frothy bloat. It is also added to many antacid, digestant and antireflux preparations, where it is claimed to relieve flatulence, coat and protect ulcer surface, aid dispersion of antacids in gastric contents, and prevent gastrointestinal reflux. However, clinical

efficacy of PDMS in such conditions is not clear. Other important uses of PDMS may include use in shampoos to make hair shiny and slippery, in food as anti-foaming agent and in contact lenses and medical devices.

Dose

For frothy bloat

Cattle : 50 – 100 ml diluted with water, PO by stomach tube or drench, or intraruminally. Dose may be repeated after 3 hours, if required.

Simethicone

Simethicone is a mixture of polydimethylsiloxane and silica gel. Similar to polydimethylsiloxane, simethicone is used orally as anti-foaming agent mainly to reduce bloating. It also decreases discomfort and pain caused by excess gas in the stomach or intestinal tract. Simethicone is not absorbed by the body into the bloodstream, and is therefore considered relatively safe.

Poloxalene

Poloxalene is a non-ionic surfactant used for treatment and prevention of frothy bloat. On oral administration, it helps to disperse the foam allowing escape of released gas. Poloxalene is administered by drench or stomach tube usually in diluted form. The direct intraruminal injection of poloxalene often does not give satisfactory results.

In recommended doses, poloxalene does not have any apparent side effect. However in food animals, 3 days withdrawal period should be followed before slaughtering the animals.

Dose

For treatment of frothy bloat

Cattle (up to 250 kg body wt.) : 25 g in 500 ml water, PO by drench or stomach tube.

(> 250 kg body wt.) : 50 g in 500 ml water, PO by drench or stomach tube.

For prevention of frothy bloat

Cattle : 22 mg/kg, PO, given 2 to 3 days before and throughout period of risk. The dose may be increased to 44 mg/kg, if required.

2. Vegetable Oils

Several bland vegetable oils (or emulsions of these oils) administered by oral route relieve acute frothy bloat. These include arachis oil, sunflower oil and soybean oil.

Arachis oil

Arachis oil (Peanut oil, groundnut oil) is pale yellow or greenish yellow oil with faint odour and bland taste. It is extracted from the seeds of *Arachis hypogaea*. It contains chiefly glycerides of oleic (50-65%) and linoleic (18-30%) acids. It closely resembles olive oil. On oral administration, it produces antizymotic, antifoaming, laxative and demulcent actions.

Dose

Cattle : 60 ml (total), PO.

Sheep : 10 – 15 ml (total), PO.

3. Volatile Oils

Turpentine oil

Oil of turpentine in a demulcent vehicle (e.g. linseed oil) has been used in frothy bloat and has shown good antifrothing property. However, this volatile oil taints flesh and milk for up to 4 to 5 days. The pharmacology of turpentine oil is given earlier in the present Chapter.

Dose

For frothy bloat

Cattle : 15 – 60 ml in 300-600 ml of raw linseed oil, PO by stomach tube or drench, or intraruminally.

Sheep : 10 ml in 60-300 ml of raw linseed oil, PO by stomach tube or drench, or intraruminally.

4. Other Drugs

In ruminants, several miscellaneous agents are used in the free gas or frothy bloat and have shown variable effects. These include sodium bicarbonate, serotonin receptor antagonists and monensin.

Sodium bicarbonate

Sodium bicarbonate (150 to 200 g) dissolved in one litre of water is given via stomach tube to cause alkalosis of ruminal contents. Sodium bicarbonate evolves CO_2 that rapidly distends the rumen and relaxes the lower oesophageal sphincter and brings about eructation of gas. However, caution should be exercised because this treatment may cause further gas production (see also Antacids, Chapter 42).

Serotonin antagonists

Serotonin receptor antagonists like ketanserin (0.1 mg/kg) and ritanserin have shown to increase the frequency of ruminal contractions and increase the volume of gas eructation in sheep. However, their clinical usefulness in the treatment of bloat is limited.

Monensin

Monensin is a metabolic product of *Streptomyces cinnamonensis* and is used primarily as a growth-promoting agent in ruminants and as anticoccidial agent in poultry. Rumen fermentation can be manipulated by dietary inclusion of low concentrations of monensin. Monensin and other ionophore antibiotics modify the rumen microflora by altering movement of ions across the biological membrane. This changes the fermentation to produce a higher proportion of propionate rather than acetate, butyrate and methane. The pharmacology of monensin has been described in Chapter 69.

OESOPHAGEAL GROOVE CLOSURE

Physiology of Oesophageal Groove

The oesophageal groove (Reticular groove) is a gutter-like invagination traversing the wall of reticulum from the cardia to the reticulo-omasal orifice. When stimulated, muscles of the groove contract forming a nearly complete tube from the cardia to the omasal canal allowing orally ingested fluids and milk to pass directly from oesophagus to abomasum, bypassing the rumen, reticulum and omasum.

Oesophageal groove closure is a reflex action with efferent impulses arriving from the brainstem through the vagus nerve. Afferent stimuli arise centrally and from receptors located in the mouth and pharynx. Anticipation of suckling involves central stimulation of oesophageal groove closure, which is considered a cephalic phase. Fluid, especially sodium-containing fluid in the pharynx, stimulates afferent fibres which reinforce the cephalic phase. The oesophageal groove has its primary function in suckling

animals and activity of the groove reflex appears to diminish after weaning and with advancing age. After about 2 years in cattle and about 1.5 years in sheep, provoked reticular groove is often irregular, incomplete or absent.

Advantages of oesophageal groove closure

Closing of oesophageal groove in ruminants offer some advantages.

a. It bypasses rumino-reticulum pathway, which allows faster and more complete absorption of drugs from abomasum than that from rumino-reticulum.
b. It increases efficacy of many drugs by not allowing their metabolism in rumen by ruminal microflora.
c. It does not allow alteration in the population of rumen microflora by antimicrobial drugs (e.g. tetracyclines, penicillins and sulphonamides), which if given directly almost invariably lead to a disruption or even elimination of ruminal microbial population.
d. It increases efficacy of several drugs administered for local intestinal effects (e.g. purgatives, antidiarrhoeals and some anthelmintics) by delivering them directly to the abomasum.

Sodium bicarbonate

In cattle, sodium salts in general and sodium bicarbonate in particular are effective in causing closure of oesophageal groove. Onset of the reflex response takes about 5 to 10 seconds and the closing of groove may remain for 60 seconds. In adult animals (>18 months of age), closure of groove by sodium bicarbonate may not be consistent and full. In sheep, sodium salts are not much useful.

Dose

Cattle : 60 ml of 10% sodium bicarbonate (or sodium carbonate) solution, PO.

Copper sulphate

Copper sulphate is occasionally used to close the oesophageal groove in cattle and sheep. A 1 percent solution of copper sulphate produces rapid and consistent closure of oesophageal groove. Its onset of action and duration is like sodium bicarbonate. Copper sulphate is an irritant and may cause itching and eczema on contact with skin. Internally it is moderately toxic and therefore should always be used with care.

Dose

Cattle : 60 ml of 1% copper sulphate solution, PO.
Sheep : 60 ml of 1-2% copper sulphate solution, PO.

Zinc sulphate

Zinc sulphate is commonly used to supply zinc in animal feeds, fertilizers and agricultural spr . Similar to copper sulphate, solution of zinc sulphate taken orally in ruminants may cause closure of oesophageal groove. It is relatively less toxic than copper sulphate, but care should be taken when zinc sulphate solution is administered internally.

Dose

Cattle : 60 ml of 5% zinc sulphate solution, PO.

Other agents

Clinical use of some dopamine receptor blocking agents such as metoclopramide and domperidone has been suggested to cause closing of oesophageal groove, but their efficacy is unclear. Antidiuretic hormone (ADH) secreted by the posterior pituitary in response to thirst and dehydration, stimulates also the oesophageal groove. Suckling is a strong stimulus for oesophageal groove closure even in adults. Therefore, it is best to allow sick calves and lambs to drink medicated milk from a nipple to assure abomasal delivery and rapid absorption. Rapid drinking from an open pail, in contrast to suckling from a nipple, frequently results in inefficient groove function and spillage of milk and fluid into the rumen. Importantly, oral administration of liquid medications to ruminants may result in spontaneous closure of the reticular groove in certain number of cases (up to 60%), which results in complete or partial rumino-reticular bypass.

MODULATORS OF RUMINORETICULAR ACTIVITY / RUMINOTORICS

Active ruminoreticular motility is an essential requirement for optimal fore-stomach functioning in ruminants. Fermentation process, absorption, passage of ingesta to omasum and abomasum, and removal of free gas by eructation are all enhanced by normal contractile activity. Since a number of nutritional, environmental, infectious and metabolic causes can lead to ruminal stasis, an important component of ruminant therapy is to promote ruminoreticular motility and to re-establish the normal rumino-reticular functions.

RUMINOTORICS

Ruminotorics (Ruminatoriums) are agents which stimulate rumino-reticular contractions and cause improvement in general functioning of rumen. These drugs are used primarily in the treatment of ruminal stasis or atony. However, none of the drug is ideal to stimulate the optimum ruminoreticular motility. Their efficacy depends on pharmacological actions of the drug, condition of the animal, and the causative factor.

1. Cholinergic Agonists

Various cholinergic agonists/parasympathomimetics such as neostigmine, physostigmine, arecoline, carbachol and bethanechol have been used as ruminotorics in veterinary medicine. All these drugs display considerable cholinergic effects and are therefore not free from adverse effects.

Neostigmine

Neostigmine is a synthetic quaternary ammonium anticholinesterase cholinomimetic agent. It increases mainly frequency of ruminoreticular contractions rather than their strength, a component particularly required in ruminal atony. The ruminoreticular stimulatory effect of neostigmine is not always reliable and some inhibition of motility due to release of catecholamines through ganglionic stimulation may occur. After oral administration, neostigmine is poorly absorbed hence is administered by parenteral route. Adverse effects of neostigmine are dose-related and cholinergic in nature. The pharmacology, side/adverse effects and other clinical uses of neostigmine have been discussed in Chapter 13.

Dose

For ruminal stasis/atony

Cattle : 0.02 mg/kg, SC.
Sheep : 0.01 – 0.02 mg/kg, SC.

Carbachol

Carbachol is a potent direct acting cholinomimetic agent having actions at both muscarinic and nicotinic receptors. It is relatively more active on the GI tract and stimulates atonic rumen. However, it tends to cause uncoordinated spastic and functionless contractions of rumen and reticulum and even paralysis of the reticulum. Spontaneous bloat may occur as a result of interference with eructation by carbachol. At high dosage, carbachol may be ineffective and in some cases may actually inhibit ruminoreticular activity. Before resorting to a potent cholinergic agent such as carbachol, considerations should be first given to more conservative approaches to stimulate rumino-reticular motility. Carbachol should not be given by IV or IM injection due to its potency. The pharmacology, side/adverse effects, clinical uses and dosages of carbachol are given in Chapter 13.

2. Prokinetics

Metoclopramide

Metoclopramide, a prokinetic, has been reported to stimulate ruminoreticular motility. While the exact mechanism for this effect is not clear, it appears that metoclopramide being a gastro-prokinetic agent sensitises also ruminoreticular smooth muscles. The pharmacology of metoclopramide has been discussed in Chapter 42.

3. Benzodiazepines

Benzodiazepines like diazepam have a transient appetite enhancing effect in some ruminants and may stimulate ruminal contractions. Their efficacy as ruminotoric agents, however, has not been evaluated much.

Dose

Diazepam

Cattle : 0.15 mg/kg, IV or IM.

Sheep : 0.05 – 0.5 mg/kg, IV.

4. Plant Alkaloids

In addition to the cholinomimetic plant alkaloids (e.g. arecoline and pilocarpine), some other vegetable alkaloids stimulate ruminoreticular motility in rumen stasis and can be used clinically for this purpose. However, their use as ruminotoric is limited mainly due to their non-specific action and/or toxic effects. These alkaloids include strychnine, veratrine and lobeline.

Dose

Strychnine

Cattle : 50 – 60 mg (total), SC.

Veratrine

Cattle : 120 – 300 mg (total), PO.

: 60 – 200 mg (total), SC.

Liquid extract of lobeline

Cattle : 4 – 30 ml (total), PO.

5. Bitters

Vegetable bitters such as gentian, nux vomica and ginger have been used in ruminotoric mixtures in attempts to stimulate salivation and perhaps ruminal contractions. An important feature of the treatment of ruminal atony is to ensure that the contents of ruminoreticulum are fluid. Bitters stimulate salivary flow to provide the fluid environment in which normal ruminoreticular function can proceed (see for detail Bitter in Appetite Stimulants).

6. Mineral Supplements

A number of co-factors are essentially required for the microbial fermentation process. These include inorganic salts and minerals like cobalt, copper, manganese, iron, zinc, calcium, magnesium, phosphorus, sulphur and iodine. Although the effectiveness of these inorganic supplements in ruminal stony is questionable, they are often included in various commercially available ruminotoric mixtures.

7. Ruminal Acidifying Agents

Depending on type of diet, the intraruminal pH may vary but is generally maintained between 6 and 7. In ruminal stasis, the intraruminal pH often increases to >7.5 because of constant inflow of bicarbonate rich saliva in absence of active ruminal fermentation and formation of volatile fatty acids (VFAs). Use of acidifying agents like acetic acid (4-5%) or vinegar returns the pH of ruminoreticular content towards physiological level. It also promotes the uptake of VFA, depresses absorption of ammonia and inhibits excessive urease activity. Lactic acid and orthophosphoric acid are also effective and safe, but must be diluted to a solution of 5%. These acidifying agents are also useful in the treatment of acute non-protein nitrogen (e.g. urea) intoxication, where elevated intraruminal pH increases activity of urease and facilitates greater absorption of free ammonia.

Dose

Vinegar or Acetic acid (5%)

Cattle : 1000 – 2000 ml, PO.

Sheep : 250 – 500 ml, PO.

8. Ruminal Alkalinising Agents

Correction of intraruminal acidosis (pH <5.0) due to grain engorgement or soluble carbohydrate overload with ruminal alkalinising agent is important in the treatment of depressed ruminoreticular motility and functions. Several alkalinising agents like magnesium hydroxide, magnesium oxide, aluminium hydroxide and carbonate, calcium carbonate and sodium bicarbonate are used to elevate a low ruminal fluid pH. The most useful ruminal antacids seem to be calcium hydroxide and magnesium carbonate. Magnesium oxide and sodium bicarbonate are more active and may cause ruminal alkalosis (see for detail Antacids). The pharmacology of some important antacids is given in Chapter 42.

Dose

Magnesium hydroxide

Cattle : 100 – 300 g (total), PO.

Sheep : 10 – 30 g (total), PO.

Magnesium oxide

Cattle : 50 g (total), PO.

Sheep : 5 g (total), PO.

Magnesium carbonate

Cattle : 10 – 80 g (total), PO.

Sheep : 1 – 8 g (total), PO.

Aluminium hydroxide or Aluminium carbonate

Cattle : 15 – 30 g (total), PO.

Sheep : 1 – 2 g (total), PO.

Calcium carbonate

Cattle : 60 – 360 g (total), PO.

Sheep : 10 – 20 g (total), PO.

Calcium hydroxide
Cattle : 10 – 80 g (total), PO.
Sheep : 1 – 8 g (total), PO.
Sodium bicarbonate
Cattle : 60 – 120 g (total), PO.
Sheep : 40 – 60 g (total), PO.

9. Other Agents

Several agents can provide relief from ruminal stasis by removing the primary cause or by facilitating the ruminoreticular motility. Opioid antagonists such as naloxone, naltrexone and diprenorphine can be used to prevent endotoxin-induced ruminal stasis. Potassium antimony tartrate can occasionally be used as ruminatorium. Antihistamines can antagonise histamine induced ruminal stasis and may promote rumino-reticular motility. An important cause of ruminal atony is pain, and analgesics may be helpful in such cases. Liquid paraffin or docusate sodium administered orally followed by gentle ruminal massage can be helpful in promoting the dissolution and passage of impacted fibrous ruminal contents, and promoting the ruminoreticular motility.

Dose
Potassium antimony tartrate
Cattle : 8 – 15 g (total), PO.
Liquid paraffin
Cattle : 1 – 2 L (total), PO.

Non-Pharmacological Approaches to Treat Ruminal Stasis

A physiological and nutritional approach to ruminant digestive disorders is much more satisfactory than a pharmacological approach. Providing a palatable balanced ration is essential for appropriate microbial fermentation. Soluble sugars such as sucrose provide a readily available energy source to stimulate ruminal movements, but these should not be used in excess otherwise they may lead to ruminal acidosis. Fresh ruminal fluid (5-10 L) is considered to be the best available ruminotoric since it contains viable ruminal bacteria and protozoa as well as many useful fermentation factors (e.g. volatile fatty acids, microbial proteins, minerals, vitamins and buffers). Water, saline and Ringer's solution may also be used to provide sufficient fluid essential for ruminoreticular fermentation.

DRUGS AFFECTING DIGESTIVE FUNCTIONS / DIGESTANTS

The digestive system is uniquely constructed to perform its specialized function of turning food into the energy. During this process, the food is first absorbed from intestine and then the absorbed food is processed to synthesise various chemicals the body needs to function. Under certain pathological conditions, the process of digestion becomes inefficient to meet the energy requirements of body. This mainly occurs due to deficiency of some essential digestants which participate in digestion of food. These may include various proteolytic, amylolytic and lipolytic enzymes produced in GI tract and/or pancreas, and bile acids supplied by liver. The deficiency of various digestants can be met exogenously and is considered useful in treating various conditions affecting the digestion.

DIGESTANTS

Digestants are a group of substances which are believed to promote digestion of food. These agents are either extracted from natural sources or synthesised chemically to perform the function of endogenous substances. Exogenous supply of digestants is beneficial only when their elaboration in the GI tract is deficient. Their routine use in tonics and appetite improving mixtures is irrational and should be avoided.

Classification

On the basis of the endogenous source, the digestants can be arbitrarily classified into three groups: pancreatic digestants, gastric digestants and hepatic digestants.

I. Pancreatic digestants and analogues
   e.g. pancreatin, pancrealipase, diastase and taka-diastase.
II. Gastric digestants and analogues
   e.g. pepsin, papain and hydrochloric acid.
III. Hepatic digestants and analogues
   a. Cholagogues
      e.g. magnesium sulphate, ceruletide, aloes, ammonium chloride and ipecacuanha.
   b. Cholerectics
      e.g. ursodeoxycholic acid, chenodeoxycholic acid, dehydrocholic acid, glycocholate, taurocholate, florantyrone, tocamphyl, clanobutin and ox bile extract.

I. Pancreatic Digestants and Analogues

The pancreas is composed of two functionally separate types of glandular tissue called exocrine pancreas and endocrine pancreas. The bulk of the pancreas is an exocrine gland (exocrine pancreas) that supplies digestive enzymes to the duodenum. A small (about 3%), but important portion of the pancreatic tissue is arranged into discrete islet of Langerhans (endocrine pancreas), which secrete hormones (e.g. insulin) into the blood stream (see Chapter 53). The exocrine pancreas is responsible for secreting an alkaline fluid containing a number of enzymes responsible for breakdown of protein, fats and carbohydrate in the small intestine. The important pancreatic enzymes include peptidases (e.g. trypsin, chymotrypsin and carboxypeptidases), lipolytic enzymes (e.g. lipases and phospholipases) and amylolytic enzymes (e.g. amylase). In certain disease conditions such as chronic pancreatitis and pancreatic insufficiency, the secretion of pancreatic juice becomes deficient, often resulting in improper digestion of proteins and fats. In such conditions, the affected animal continues to lose weight rapidly despite having good appetite because its fat and glycogen reserves and muscle proteins are drawn on to provide the energy necessary to maintain the vital processes. Animals suffering from these conditions can be maintained by supplementing pancreatic enzymes or their analogues from exogenous sources.

The enzymes of pancreas are available in preparations known as pancreatin and pancrelipase. They contain principally amylase, proteases and lipase and are used in patient suffering from pancreatic insufficiency in conditions like cystic fibrosis, pancreatic duct obstruction, chronic pancreatitis and after pancreatectomy.

Pancreatin

Pancreatin, also known as pancreatinum, is an alcoholic extract of the pancreas of hog or ox. It is available as a cream coloured

amorphous powder and contains principally amylase, proteases and lipase. It has a faint characteristic, but no offensive odour. It contains not less than 1 unit of protease (trypsin), 15 units of lipase and 12 units of amylase activity per mg of powder. Numerous formulations (e.g. tablets, capsules, powders and granules) of pancreatic extracts are available, some of which are enteric-coated and their enzyme contents and bioavailability vary widely.

Pharmacological effects

Pancreatin contains pancreatic enzymes and shows greatest activity in neutral or slightly alkaline solution and is inactivated in acidic medium. So its enteric-coated preparations are considered better than uncoated preparations because they are less likely to be destroyed in the stomach. On oral administration, pancreatin provides the pancreatic enzymes to catalysis hydrolysis of fats, proteins and starch. It also decreases intraductal pressure and relieves pain in chronic pancreatitis. It can reduce fat and nitrogen content of stools and prevent pancreatic insufficiency induced by diarrhoea and steatorrhoea.

Pharmacokinetics

The pancreatic enzymes remain unabsorbed from GI tract after oral administration of pancreatin. Onset of action starts in about 30 minutes and peak therapeutic effect occurs in 2 to 5 hours.

Side effects/Adverse effects

In recommended doses, adverse effects of pancreatin are few. In high dosage, it may cause nausea and diarrhoea. The high purine contents of the pancreatic digestant may also result in dose-related hyperuricaemia, uric acid crytalluria and urolithiasis.

Clinical uses

Pancreatin is used in the treatment of diarrhoea and weight loss due to excessive pancreatic insufficiency (EPI) in dogs and cats. In refractory cases, simultaneous administration of non-systemic alkalinising agents or cimetidine given 30 minutes before dosing may be used as an adjunct to pancreatic therapy. Cimetidine reduces gastric acid production thereby decreasing inactivation of pancreatin. Dietary control is often essential for the successful management of animals with exocrine pancreatic insufficiency. Frequent small amounts of a diet containing low fat, low fibre, high digestible carbohydrate and high quality protein are useful.

Dose

The pancreatin dose in small animals is adjusted according to the response of patient. Addition of two 5 ml teaspoonful of powdered non-enteric coated preparations with each meal per 20 kg of body weight is generally considered an effective starting dose. Two meals a day are usually sufficient to promote weight gain and to resolve diarrhoea within 4 to 5 days. As soon as clinical improvement becomes apparent, the dose can be decreased to at least one teaspoonful of enzyme supplement per meal.

Note: Commercial dried pancreatic extracts (pancreatin) are expensive; therefore, use of 100-120 g per 20 kg body weight of chopped raw ox or pig pancreas obtained from certified slaughterhouse is an economical alternative. Pancreas, if used, can be stored frozen at –20° C for at least 3 months, without much loss of enzyme activity.

Pancrelipase

Pancrelipase contains pancreatic enzymes including lipase (mainly), amylase and protease. It is obtained from the pancreas of hog. When compared on a weight basis, pancrelipase has at least 4 times the trypsin and amylase content of pancreatin and at least 12 times the lipolytic activity of pancreatin. The enzymes found in pancrelipase help to digest and absorb fats, proteins, and carbohydrates. Its properties, action, adverse effects and uses are like pancreatin.

Dose

Dogs : 1 – 2 teaspoonful of pancrelipase powder mixed with each meal at least 3 times per day. The drug is mixed with food thoroughly and allowed to stand for 15-20 minutes before feeding. Dosage should be adjusted as necessary.

Cats : 0.5 – 0.75 teaspoonful mixed with each meal. The dosing schedule is same as mentioned for dogs.

Diastase

Diastase is an amylase or a mixture of amylases which catalyse the breakdown of starch into maltose. It is generally extracted from malt and exists as a yellowish white amorphous powder. It is most active in solutions which are approximately neutral; a reaction of pH 4 destroys the enzyme. It is capable of converting 50 times its weight of starch into sugars. Diastase is used occasionally in pancreatic insufficiency for replacement of pancreatic amylase. It is also used for the treatment of starchy dyspepsia with hyperacidity.

Dose

Dogs : 0.06 – 0.3 g (total), PO.

Taka-diastase

Taka-diastase is a form of diastase (amylolytic enzyme) obtained from fungus *Aspergillus oryzae*. Takadiastase is named after Jokichi Takamine, who developed the method first used for its extraction. It occurs as yellowish white powder and converts 500 times its weight of starch into sugars. It is indicated in dyspepsia and debility.

Dose

Dogs : 0.06 – 0.3 g (total), PO.

II. Gastric Digestants and Analogues

Pepsin

Pepsin is a proteolytic enzyme collected from the glandular layer of fresh stomach of the hog. It occurs as an amorphous translucent colourless or light yellowish powder possessing slight acid or saline taste. It is active only in acidic medium and looses activity in alkaline pH. It can digest up to 2500 times its weight of proteins into peptones. Pepsin has no action on fat and starch. Pepsin is administered to assist gastric digestion. It should preferably be given after meals, followed by a dose of dilute hydrochloric acid (0.4%). It is mainly used in indigestion, gastric achylia due to atrophic gastritis, gastric carcinoma and pernicious anaemia.

Dose

Dogs : 0.3 – 0.6 g (total), PO.

Foals & calves : 2.6 – 4 g (total), PO.

Papain

Papain, also known as papaya proteinase I, is a cysteine protease enzyme obtained from raw papaya i.e. *Carica papaya*.

Papain is sometimes refereed to 'vegetable pepsin' because it contains proteolytic enzymes resembling pepsin. Although differing in strength in accordance with the method of manufacturing, papain is capable of digesting 35 times its weight of lean meat. Unlike pepsin, papain acts in acid, neutral or alkaline medium. It is used as a digestant because it has actions much similar to those of pepsin. It is also employed as a mucosolvent because of its liquefying effects on excessive mucus in the mouth and stomach.

Dose

Dogs : 0.3 – 0.6 g (total), PO.

Hydrochloric acid

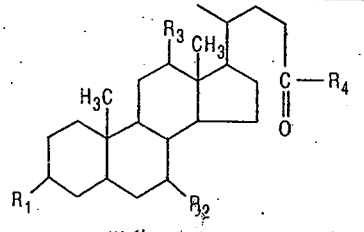
Dilute hydrochloric acid can be used as a replacement for gastric juice in achlorhydria. About 2 to 5 ml of dilute HCl (10%) should be further diluted to 50-100 ml with water and administered during meals by stomach tube to avoid dissolution of tooth enamel. Dilute hydrochloric acid also has antizymotic and intestinal antiseptic actions.

III. Hepatic Digestants and Analogues

One of the several functions of liver is that of a secretory gland of the digestive system. It secretes bile that has an important role in the fat digestion. Bile acids and their salts promote the digestion of fats and the absorption of long-chain fatty acids and fat-soluble vitamins.

Physiology of Bile Acids

Bile acids are steroid acids found predominantly in the bile of mammals. Bile acids are synthesised from cholesterol in a series of steps, which in mammals are mainly trihydroxycholic acid (cholic acid) and dihydroxy chenodeoxycholic acid (chenodeoxycholic acid). After their synthesis, these primary bile acids are conjugated with glycine and/or taurine, or with sulphate or glucuronide, and are then stored in the gallbladder. The bile acids are secreted into the bile canaliculi compounded with a cation, usually sodium, in the form of bile salts. Once secreted, bile acids are largely absorbed in the ileum and recycled via an enterohepatic cycle. Only 5-10% of the secreted bile acids are lost in the faeces during one enterohepatic cycle, the minimal loss being replenished by hepatic synthesis. Bile acids can also undergo structural modifications (i.e. deconjugation and dehydroxylation) by the intestinal microflora forming the secondary bile acids, of which deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid are important. Additionally, bacterial and hepatic metabolism can lead to formation of ursodeoxycholate from chenodeoxycholic acid. The chemical structures of major bile acids are given in Figure 44.7.



Basic Structure

Bile acid	R_1	R_2	R_3	R_4
Cholic acid	– OH	– OH	– OH	Glycine, taurine, or – OH
Chenodeoxy cholic acid	– OH	– OH	– H	
Deoxycholic acid	– OH	– H	– OH	
Lithocholic acid	$-SO_3^-$ /– HO	– H	– H	
Ursodeoxycholic acid	– OH	– OH	– H	

Fig. 44.7. Chemical structures of major bile acids.

The gallbladder mainly stores and concentrates bile during periods between feeding. When food, especially fat containing food, reaches duodenum, the cholecystokinin (CCK) is released from the GI endocrine cells. It causes opening of sphincter of Odii present at union of common bile duct and duodenum and produces contraction of gall bladder. When the fats are digested, the secretion of CCK ceases, sphincter of Odii closes, and bile is diverted to the gall bladder. Bile production is enhanced by hormone secretin and stimulation of vagus nerve.

a. Cholagogues

Cholagogues are agents which promote flow of bile into the intestine, especially as a result of contraction of the gall bladder. Dietary fat and concentrated magnesium sulphate (or sodium sulphate) introduced directly into the duodenum through a tube exert a cholagogue effect through release of CCK-pancreozymin from the upper small intestine. Ceruletide, a decapeptide isolated from the skin of the Australia frog, has all properties of gastrin and CCK-pancreozymin in mammals and is used in humans to contract the gall bladder during cholecystography. Several other non-specific agents like aloes, ammonium chloride, ipecacuanha and mercurous chloride possess this activity but have no therapeutic use. Vagal stimulation will also promote contraction of the gall bladder. Overall, the use of cholagogues in veterinary medicine is limited.

b. Cholerectics

Cholerectics are agents which stimulate liver to increase output of bile. Drugs which stimulate liver to increase the output of bile of low specific gravity are called hydrocholerectics. A number of natural bile salts and partially synthetic derivatives are used therapeutically as cholerectics. Bile salts used therapeutically have duel action in directly promoting fat absorption and stimulating biliary secretion after they have been absorbed. In human beings and small animals, some agents (e.g. ursodeoxycholic acid and chenodeoxycholic acid) are used as adjunctive therapy for the medical management of dissolution of cholesterol containing gallstones. Although certain individual bile acids (e.g. lithocholic acid) are toxic and have shown to produce cholestasis, others (e.g. ursodeoxycholic acid) have shown to provide therapeutic benefits in cholestatic liver disease.

Ursodeoxycholic Acid/Ursodiol

Ursodeoxycholic acid (UDCA), also known as ursodiol, is one of the naturally occurring secondary bile acids, which are metabolic by-products of intestinal bacteria. It is a relatively hydrophilic bile acid. It is used in the treatment of a variety of chronic hepatobiliary disorders in dogs, cats and humans.

Mechanism of action

The exact mechanism by which ursodeoxycholic acid produces beneficial effects is not clearly understood. It seems that ursodeoxycholic acid may exert beneficial therapeutic effects via different mechanisms.

a. UDCA decreases cholesterol content of bile by inhibiting its intestinal absorption, and by suppressing its hepatic

synthesis and secretion. A high cholesterol : bile salt ratio favours crystallisation of cholesterol in bile, which leads to cholesterol stone (cholelith) formation. Although cholelithiasis is rather uncommon in dogs, ursodeoxycholic acid by reducing cholesterol saturation in bile decreases gallstone formation and promotes its dissolution by promoting mobilisation of cholesterol from gall stones by the formation of a liquid-crystalline phase. It has no effect on calcified stones or bile pigment stones.

b. UDCA protects hepatocytes by displacing toxic hydrophobic bile salts. Because the accumulation of toxic hydrophobic bile acids (e.g. lithocholic acid and other monohydroxy bile acids) in liver is believed to be responsible for additive liver cells injury and inflammation in cholestatic liver diseases, the UDCA therapy may lead to replacement of the increased concentration of toxic endogenous bile acids by a relatively non-toxic hydrophilic ursodeoxycholic acid. Ursodeoxycholic acid is also reported to inhibit absorption of other toxic bile acids.

c. UDCA is an unusual bile acid because it produces bicarbonate-rich hypercholeresis (enhanced bile flow) with increased bile pH. It is possible that the increased bile flow of high pH may also be involved in promoting biliary excretion of potentially toxic cholephilic agents (primarily acidic agents because of an increased bile pH).

d. UDCA seems to have immunomodulating effect under certain conditions and this effect may contribute to the non-specific beneficial effects observed in hepatobiliary diseases.

Pharmacokinetics

Ursodeoxycholic acid is well absorbed from the small intestine after oral administration. After absorption, it is conjugated with either taurine or glycine in liver and secreted into the bile. Only very small quantity enters the systemic circulation and excreted in urine. After each entero-hepatic cycle, some portion of drug is metabolised by bacterial degradation and is excreted in the faeces.

Side effects/Adverse effects

Most of the present information on the toxicity of bile acids is based on studies in humans and experimental animals. Although some species differences have been observed, but in general, ursodeoxycholic acid is well tolerated and is considered virtually non-toxic. Diarrhoea and other GI effects have rarely been noted in humans taking UDCA. While ursodeoxycholic acid may be useful in treating patients with chronic liver disease, some patients may experience further impairment of bile acid metabolism. The drug should not be used in extra-hepatic biliary obstruction.

Clinical uses

In small animals, ursodeoxycholic acid may be beneficial as adjunctive therapy for the management of cholesterol containing gallstones (size <15 mm in diameter) and/or in patients with chronic liver diseases, particularly where cholestasis (bile toxicity) plays an important role. As the dissolution of stone is a very slow process (may take 1-2 years) and recurrences are common once the treatment is discontinued, repeat courses of ursodeoxycholic acid may have to be given.

Dose

Dogs & cats : 10 – 15 mg (total), PO, once daily.

Chenodeoxycholic acid

Chenodeoxycholic acid (also known as chenodesoxycholic acid, chenocholic acid or chenodiol) is a primarily bile acid used in the treatment to cholelithiasis. It is synthesised in liver and secreted as bile salt in intestine, where it is converted by colon bacteria to secondary bile acid known as lithocholic acid. Salts of chenodeoxycholic acid are called chenodeoxycholates. Chenodeoxycholic acid acts primarily by inhibiting cholesterol synthesis and does not affect intestinal cholesterol absorption much. Unlike ursodeoxycholic acid, it acts primarily by inhibiting 3-hydroxymethyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), the rate limiting enzyme in hepatic cholesterol synthesis. In addition, it enters the bile and causes solubilisation of cholesterol.

Chenodeoxycholic acid is more toxic then ursodeoxycholic acid, especially in dogs where it is not a major bile acid. In human beings, therapeutic doses of chenodeoxycholic acid produce diarrhoea and rise in plasma transaminase activity and serum cholesterol in low-density lipoprotein (LDL) fraction. In primates, it causes foetal abnormalities at doses slightly higher than those utilised clinically; so it contraindicated in pregnant animals. As a result of side effects, therapy with chenodeoxycholic acid alone is not recommended. Chenodeoxycholic acid is combined usually with ursodeoxycholic acid, where due to different mechanisms of action the combination gives better result than chenodeoxycholic acid alone.

Dehydrocholic acid

Dehydrocholic acid is a synthetic bile acid, manufactured by the oxidation of natural cholic acid. Unlike most physiological bile acids and their conjugates, dehydrocholic acid does not increase biliary secretion of phospholipids and cholesterol and may decrease bilirubin excretion. It therefore produces hydrocholeresis i.e. increases the volume and water content of the bile from liver without increasing the amount of solids. This is considered useful in improving biliary drainage and in flushing out bile sludge. The hydrocholeretic effect of dehydrocholic acid occurs mainly due to its inability to form micelles (small aggregates of bile acids, fats and phosopholipids necessary for normal fat absorption). After administration, dehydrocholic acid is extensively metabolised and some of metabolites may form micelles. Due to this reason, dehydrocholic acid should not be used for conditions requiring dissolution of stones.

Dehydrocholic acid is used mainly in humans to prevent bile sludging and to improve biliary drainage. It has also been recommended in cats with cholangiohepatitis. Dehydrocholic acid is also a stimulant laxative.

Dose

Dogs & cats : 10 – 15 mg/kg, PO, 3 times daily.

Cattle : 3 g, IV.

Other drugs

Naturally occurring bile acid conjugates such as glycocholate (conjugate of cholic acid with glycine) and taurocholate (conjugate of cholic acid with taurine) enhance bile flow to a lesser extent. Florantyrone and tocamphyl, the synthetic products, are also available as cholerectic agents. Several oxybutyric acid derivatives such as clanobutin and methoxynaphthalene-oxybutyric acid are used in veterinary medicine as cholerectic agents and are believed to enhance digestion. Ox bile extract is also used as cholerectic agent. It is prepared by partial evaporation of fresh ox bile and contains sodium salts of ox bile acids equivalent to not less than 45 percent of cholic acid.

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SECTION-VIII

DRUGS ACTING ON RESPIRATORY SYSTEM

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CHAPTER 45 EXPECTORANTS AND ANTITUSSIVES

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- Expectorants
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Centrally acting antitussives
- Opioid/Narcotic antitussives
- Non-opioid/Non-narcotic antitussives

Respiratory system performs several functions. Most importantly, it delivers oxygen to the cardiovascular system for redistribution to the body and then removes carbon dioxide. In addition, the respiratory system maintains acid-base balance, acts as blood reservoir and metabolises some bioactive substances. The upper airways also provide for sense of smell and play a role in temperature regulation in panting animals. The airway epithelial cells also generate secretory immunoglobulins (IgA), collectins (including Surfactants A and D), defensins and other peptides and proteases, reactive oxygen species, and reactive nitrogen species. Although the anatomy of respiratory tract differs markedly among species, but regulation of specific aspects of homeostasis at the cellular level in all species requires a close integration of nervous, pulmonary and circulatory systems.

Various disease processes which affect the respiratory system generally impede ventilation and alveolar gas exchange in different ways. The respiratory disorders like pneumonia, bronchitis, sinusitis, rhinitis and emphysema differ in their aetiology but have one common characteristic that they obstruct airway and block effective pulmonary ventilation. The specific objective of therapeutic agents, therefore, is to maintain the adequate oxygen supply. This can be accomplished by modifying the volume and viscosity of the secretion, bronchodilation, controlling infection, decongestion, suppressing excessive unproductive coughing and minimising inflammation.

EXPECTORANTS

Expectorants (Mucokinetics) are agents which increase volume or fluidity of secretions in the respiratory tract and facilitate their removal by ciliary action and coughing. Healthy cilia, adequate volumes of respiratory tract secretions and a functional cough are necessary to effectively clear airway mucus. However, many diseases like tracheobronchitis, bronchopneumonia and chronic obstructive pulmonary disease can disturb the normal reflexes of respiratory tract and promote retention and inspissations of respiratory tract mucus. In such conditions, several pharmacological agents have been used to mobilise and effectively evaluate exudates which impair ventilation.

Expectorants can increase the fluidity of respiratory secretions through several mechanisms. In presence of expectorants, especially in the acute inflammatory stage where mucosa is dry and inflamed, cough is less frequent but more productive. In the recovery stages of inflammation where there is excess of viscous mucus and tissue debris, use of expectorants would also be beneficial. The clinical usefulness of expectorants is still obscure, but these are almost always added as ingredient in cough medications and are used frequently to treat respiratory disorders in animals.

Classification

Depending on their mode of action, expectorants can be divided into following important groups:

I. Secretory expectorants/Stimulant expectorants
   1. Reflex acting expectorants
      e.g. ipecacuanha, balsam of Tolu and squill.
   2. Direct acting expectorants
      e.g. guaiacol, guaifenesin, volatile oils and terpin hydrate.
   3. Mixed acting expectorants
      e.g. potassium iodide, ammonium chloride, ammonium carbonate and sodium nitrate.

II. Mucolytic expectorants
   e.g. bromhexine, dembrexine, ambroxol, acetylcysteine, carbocysteine, erdosteine and dornase alfa.

III. Diluent expectorants
e.g. water aerosols (steam), saline aerosols, propylene glycol and glycerine.

IV. Miscellaneous expectorants
e.g. carbon dioxide, antimony potassium tartrate and syrup.

I. SECRETORY EXPECTORANTS / STIMULANT EXPECTORANTS

Secretory or stimulant expectorants are primarily mild irritants. The exact mechanism by which these drugs increase the volume and fluidity of respiratory secretions is not understood, but they are postulated to act either directly through activating the submucosal glands or indirectly through stimulation of the gastropulmonary vagal reflex.

1. Reflex Acting Secretory Expectorants

A number of substances mainly of plant origin which produce nausea and emesis at higher doses are occasionally used at lower doses to stimulate the vagus to produce reflex secretion by the tracheobronchial glands.

Ipecacuanha

Ipecacuanha (Ipecac), obtained from the dried root and rhizome of *Cephaelis ipecacuanha*, is used primarily as an emetic (see for detail Chapter 44). However at doses much lower than those required for emesis, it stimulates respiratory tract secretions by reflex action mediated by autonomic (especially vagus) pathways. At recommended doses ipecacuanha rarely exhibits toxic effects. At higher doses it may stimulate lachrymation and salivation and produce other adverse effects. As an expectorant, ipecacuanha is very less used in veterinary medicine.

Dose

Ipecacuanha powder

Dogs : 15 – 20 mg (total), PO.

Cattle & horses: 4 – 10 g (total), PO.

Liquid extract of ipecacuanha (contains 2% of emetine)

Dogs : 0.06 – 0.2 ml (total), PO.

Cattle & horses: 2 – 4 ml (total), PO.

Balsam of Tolu

Balsam of Tolu (Tolu balsam) is a brown or yellowish brown semi fluid or nearly solid resinous mass obtained from the trunk of *Myroxylon balsamum* (*Toluifera balsamum*), a tree native to South America. The resin as well as the leaves and fruit have been traditionally used by the native pre-columbian people, who inhabited the same place where now is located a small town of Tolu in Northern Columbia. The Tolu balsam is soluble in alcohol, ether, chloroform and fixed alkali solutions. Balsam of Tolu is rich in free aromatic acids and contains free cinnamic acid (12-15%), free benzoic acid (8%) and free esters as benzyl benzoate. It also contains numerous triterpenoids. It appears to induce respiratory tract secretions, probably via a gastropulmonary reflex. In addition, some direct stimulant action on tracheobronchial glands has been suggested. Balsam of Tolu is a common ingredient of cough mixtures to which it is added in the form of syrup or tincture.

Dose

Powder of balsam of Tolu

Dogs : 0.3 – 2 g (total), PO.

Cattle & horses: 30 – 60 g (total), PO.

Tincture of balsam of Tolu (10%)

Dogs : 1 – 2 ml (total), PO.

Other drugs

Several other reflexly acting plant preparations such as balsam of Peru, acalypha, squill and cocillana are available and used occasionally as expectorants in bronchitis. The balsam of Peru (Peru Balsam) is an aromatic resin extracted from the variant *Myroxylon balsamum pereirae*, a native from Central America. Acalypha plant with several species is distributed widely from southern United States to Uruguay, and squill (Scilla) is distributed throughout Europe and Asia. Cocillana is the dried bark of *Guarea rusbyi*, a Bolivian tree.

2. Direct Acting Secretory Expectorants

Direct acting secretory expectorant probably stimulate the tracheobronchial glands directly and produce an associated active hyperaemia in the respiratory tract. Many of these agents are aromatic compounds which probably are eliminated in part by the respiratory mucosa.

Guaiacol and Guaifenesin

Guaiacol is a naturally occurring organic compound with the formula $C_6H_4(OH)(OCH_3)$. Guaiacol is obtained by fractional distillation of guaiacum or wood creosote, or is synthetically prepared. It is available as an oily liquid or crystalline mass, which has unpleasant odour and taste. It is poorly soluble in water and highly soluble in glycerine, alcohol and ether. Guaifenesin (Guaiphenesin, glyceryl guaiacolate) is a glyceryl ether of guaiacol (Figure 45.1). Although both guaiacol and guaifenesin have been used for their expectorant effects, the use of guaifenesin is more frequent in therapeutics.

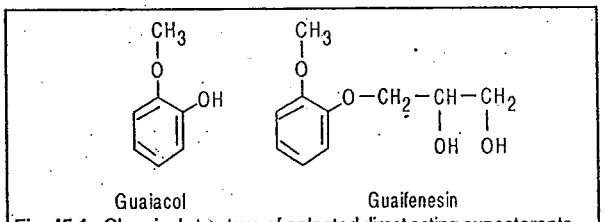


Fig. 45.1. Chemical structure of selected direct acting expectorants.

Guaiacol and guaifenesin are believed to directly increase bronchial secretion and mucosal ciliary action, when after absorption the tracheobronchial glands secrete them. It is suggested that these agents also reduce the stickiness of mucus in chronic bronchitis favouring airway particle clearance. Although the main use of guaifenesin in medicine is as central muscle relaxant (see Chapter 16), it forms an important ingredient of many commercially available expectorant mixtures. Guaifenesin also has other known neurological effects, including an analgesic effect that is related to its action as a skeletal muscle relaxant, and possible inhibition of platelet aggregation. It is also widely used in veterinary medicine to induce and maintain anaesthesia in horses and some other species. Nausea, vomiting and dry mouth are important side effects seen generally after overdosages.

Dose

Guaiacol

Dogs : 0.12 – 0.6 ml (total), PO.

Cattle : 8 – 15 ml (total), PO.

Horses : 2 – 6 ml (total), PO.

Table 45.1: Selected volatile oils and balsamic resins used as secretory expectorants.

Compound	Nature	Source	Remarks
1. Benzoin	Balsamic resin	Incised stem of *Styrax benzoin.*	Should not be used for longer time due to irritant action.
2. Camphor	Stearotene (solid volatile oil)	Synthetic or from stem of *Cinnamomum camphora.*	– do –
3. Menthol	Stearotene (solid volatile oil)	Synthetic or from oil of peppermint.	– do –
4. Oil of eucalyptus	Volatile oil	Fresh leaves of *Eucalyptus dumosa* and other *Eucalyptus* spp.	– do –
5. Oil of lemon	Volatile oil	Fresh peel of fruit *Citrus lemon.*	– do –
6. Oil of pine	Volatile oil	Wood of *Pinus palustries* and other *Pinus* spp.	– do –
7. Oil of turpentine	Volatile oil	Crude turpentine (oleo-resin) collected from *Pinus palustries* and other species of pines.	– do –
8. Terebene	Rectified oil of turpentine	Turpentine oil obtained from *Pinus* spp. and rectified by distillation from aqueous sodium hydroxide.	Less irritant than oil of turpentine.
9. Terpene hydrate (terpinol)	Derivative of oil of turpentine	Formed by action of nitric acid on rectified oil of turpentine (terebene) in the presence of alcohol.	Less irritant than oil of turpentine and more useful for chronic respiratory diseases.

Volatile oils and Balsamic resins

Many volatile oils or their derivatives and resin-containing balsams are traditionally used as expectorants. These agents are believed to increase secretion of respiratory tract by stimulating the tracheobronchial glands directly. They also induce respiratory tract hyperaemia and hasten repair due to their mild irritant action. These agents are used primarily as medicated vapour inhalation, but can also be used by oral route occasionally. Some volatile oils are toxic and may produce gastrointestinal or urinary irritation, if used in higher dosages. The frequently used compounds in various cough remedies are given in Table 45.1.

3. Mixed Acting Secretory Expectorants/Saline Expectorants

Several inorganic and organic salts appear to stimulate the gastropulmonary vagal reflex with subsequent activation of submucosal tracheobronchial glands. The most frequently used mixed acting secretory expectorants are potassium iodide, calcium iodide, sodium iodide, ethylenediamine dihydroiodide, ammonium chloride and carbonate, and sodium and potassium nitrate. These are generally called saline expectorants.

Potassium iodide

Potassium iodide (KI) is white granular or colourless and transparent crystals having saline and bitter taste. It is soluble is water, alcohol and glycerine. On oral administration, it increases respiratory tract secretions by about 150% by both reflex action originating from gastric irritation and direct stimulation of respiratory glands. The iodide is rapidly excreted through the bronchial glands and in this process irritates them, increasing the volume of secretion. In addition, a mucolytic effect of potassium iodide has been suggested causing thinning of bronchial secretions.

The major medicinal uses of KI are as a nutritional supplement in animal feeds, as expectorant to increase water content of respiratory secretions and encourage effective coughing, and in emergency treatment for hyperthyroidism (see Chapter 50). It is also used topically for the treatment of sporotrichosis, erythema nodosum and some other dermal conditions. Main adverse effects of iodide ingestion include iodinism with induction of goitre and hypothyroidism after prolonged (months) use. Potassium iodide should not be given to patients sensitive to iodide, acute inflammatory conditions of respiratory tract and in pregnant animals. Iodides should not be administered to animals whose milk is used for human consumption. It is more beneficial in the later stages of bronchitis where it causes liquefying or loosening action on thick tenacious mucus. In early acute bronchitis, it may irritate coughing due to its irritant action on the mucosa.

Dose

Dogs : 0.2 – 0.6 g (total), Po.
Cats : 0.06 – 0.12 g (total), PO.
Horses : 4 – 8 g (total), PO.
Cattle : 8 – 16 g (total), PO.

Ammonium chloride and Ammonium carbonate

Ammonium chloride and ammonium carbonate are saline expectorants, still widely used in cough remedies. These are primarily gastric irritants, which reflexly enhance bronchial secretions. Direct irritant action on tracheobronchial glands has also been suggested. Ammonium salts are irritant to the gastric mucosa and may induce nausea and vomiting. In high dosages, ammonium salts may produce hyperammonemia, which as characterized by dyspnoea, muscle fasciculation and convulsions which may terminate in acute pulmonary oedema. Due to their doubtful efficacy and toxicity, use of ammonium salts as expectorants is declining in recent time.

Dose

Ammonium chloride

Dogs : 50 mg/kg, PO, 2 times daily.
Horses : 4 – 15 g (total), PO, 1 to 2 times daily.

II. MUCOLYTIC EXPECTORANTS

Mucolytic expectorants (Mucolytics) are agents which decrease viscosity of thick mucus and hence facilitate its removal

by ciliary action and expectoration. As the viscosity of mucous secretions in the respiratory tract is dependent upon the concentrations of mucoproteins, these agents decrease viscosity of mucus by producing depolymerisation of glycoprotein molecules mainly the mucopolysaccharide (glycosaminoglycan) strands. Although their major action is not that of increasing the volume or fluidity of secretions, they are often regarded as expectorants and are widely used for this purpose in chronic inflammatory conditions. The rationale for their use is that mucus of lower viscosity is more easily carried up the tracheobronchial tree by the mucociliary clearance mechanisms.

Bromhexine

Bromhexine is a synthetic derivative of the alkaloid vasicine obtained from *Adhatoda vasica* (Vasaka). Bromhexine is a potent mucolytic agent capable of inducing thin copious bronchial secretion. It depolymerises mucopolysaccharides directly as well as by liberating lysosomal enzymes causing breakdown of network of fibres in the tenacious sputum. It is particularly useful if mucus plugs are present. Bromhexine or its active metabolite also stimulates synthesis and release of surfactants by type II pneumocytes, which act as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall. Bromhexine results in an increase in immunoglobulin levels in airway secretions probably by enhancing membrane permeability. All these actions promote mucus clearance, facilitate expectoration and ease productive cough, allowing animals to breathe freely and deeply.

Bromhexine may be administered by both oral and parenteral routes and has been used as ancillary therapy in the management of chronic bronchitis in dogs, bronchopneumonia in cattle, horses and pigs, and chronic coughing in horses. It is also used for the treatment of amniotic fluid aspiration in newborn calves and piglets. It is available in a solution or as a powder used either alone or mixed with antibacterial drugs. Its main side effects are rhinorrhoea and lachrymation.

Dose

Dogs & cats	:	1 mg/kg, PO or IM, 2 times daily.
Piglets	:	0.3 – 1.5 mg/kg, PO or IM, once daily.
Calves	:	0.5 – 1 mg/kg, PO or IM, once daily.
Horses	:	0.1 – 0.25 mg/kg, PO or IM, once daily for 7 days.
Cattle & pigs	:	0.2 – 0.5 mg/kg, PO or IM, once daily.

Dembrexine

Dembrexine is a mucolytic expectorant used primarily in equines. Dembrexine enhances serous glandular secretions by its action on serous glandular cells in respiratory mucosa and diminishes viscosity of tracheobronchial mucus. Such mucus of modified composition and deceased viscosity is more amenable to mucociliary clearance. Dembrexine is rapidly and almost completely absorbed after oral administration. It is also rapidly eliminated from the plasma but undergoes enterohepatic circulation. Dembrexine has lower degree of toxicity and is well tolerated by animals. It is recommended in respiratory diseases where excess or tenacious mucus in present in the airways. It also possesses some antitussive action.

Dose

Horses	:	0.3 – 0.5 mg/kg, PO, 2 times daily for 10 days. Treatment is generally initiated by an IV loading dose followed by oral maintenance dose for up to 15 days.

Ambroxol

Ambroxol is a metabolite of bromhexine. Its mucolytic and secretolytic actions are similar to those of parent drug bromhexine. It also possesses some anti-inflammatory and local anaesthetic actions which help to provide pain relief in acute sore throat. Ambroxol is used in the treatment of respiratory diseases associated with viscid or excessive mucus.

Acetylcysteine

Acetylcysteine, also known as N-acetylcysteine or N-acetyl-L-cysteine, is a derivative of amino acid L-cysteine. It is used mainly by inhalation for respiratory mucolytic action and by intravenous or oral route for the treatment of paracetamol overdose.

Properties

Acetylcysteine occurs as a white crystalline powder with slight acetic odour and is freely soluble in water and alcohol. Acetylcysteine is incompatible with oxidizing agents and liberates hydrogen sulphide when exposed to rubber, copper, iron (not stainless steel, glass or plastics), and during autoclaving. Therefore, aerosol devices which do not permit contact of drug with rubber or metal parts of the equipment are mostly used to administer drug solution.

Pharmacological effects

When administered into respiratory tract in the form of spray or aerosol, acetylcysteine reduces the viscosity of both purulent and non-purulent secretions and expedites removal of these secretions via coughing, suction or postural drainage. It is believed that the sulphydryl groups of acetylcysteine breaks the disulphide bonds in glycoproteins in exudates and so changes the viscosity of mucus. In addition, acetylcysteine serves as a precursor of glutathione, a major scavenger of free oxygen radicals associated with inflammation. The drug also appears to induce respiratory tract secretions, probably via a gastropulmonary reflex. The mucolytic properties of acetylcysteine are best shown when drug is administered at a concentration of 10-20% and pH 7-9.

Pharmacokinetics

When given orally (for paracetamol toxicity), acetylcysteine is absorbed from the GI tract and extensively distributed to liver, kidneys and lungs, where it may accumulate. It is metabolized rapidly to cysteine and cystine in the liver. When administered via nebulisation or intratracheally into pulmonary tract, most of the drug is involved in the mucolytic effect and the remainder is absorbed and follow the same course as seen after oral administration.

Side effects/adverse effects

When administered into the pulmonary tract, acetylcysteine hypersensitivity has been reported in few cases. It may cause bronchial irritation, bronchoconstriction, ciliary inhibition and coughing, especially in patients suffering from asthma. This drug-induced bronchoconstriction can be avoided by simultaneous administration of a bronchodilator. When given orally for paracetamol toxicity, acetylcysteine can cause GI effects like nausea and vomiting and rarely urticaria. Acetylcysteine has very

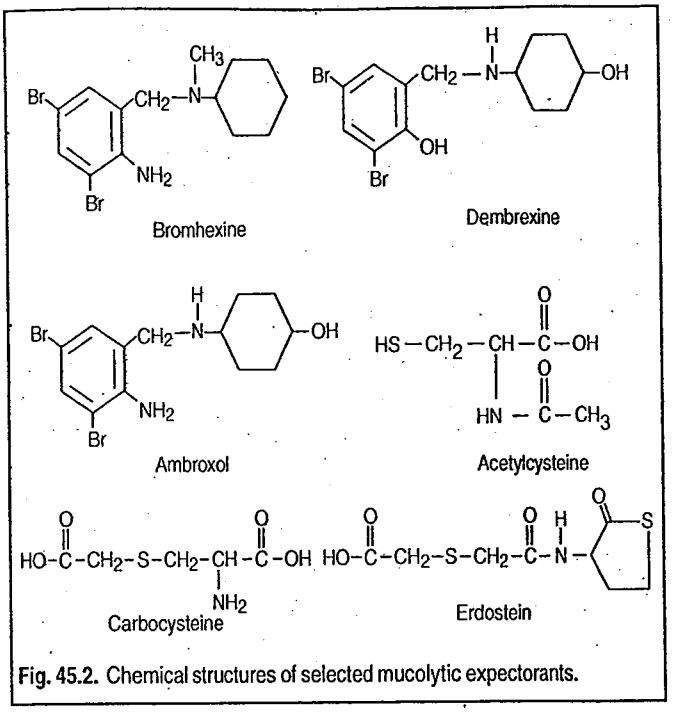


Fig. 45.2. Chemical structures of selected mucolytic expectorants.

bad taste hence use of taste masking drugs have been suggested. In animals, usually gastric or duodenal tubes may be necessary.

Contraindications and precautions

Because acetylcysteine is metabolized to sulphur containing products, it should be used cautiously in animals suffering from liver disease characterised by hepatic encephalopathy.

Drug interactions

Antibiotics like penicillins and tetracyclines are inactivated by acetylcysteine and should not be mixed together for aerosol administration.

Clinical uses

Acetylcysteine is used in both human and veterinary medicines as a mucolytic agent for the treatment of respiratory diseases. Inhaled acetylcysteine is indicated as an adjuvant in respiratory conditions with excessive and/or thick mucus production such as emphysema, bronchitis, tuberculosis, bronchiectasis, amyloidosis, pneumonia, cystic fibrosis and Chronic Obstructive Pulmonary Disease (COPD). It is also used post-operatively and in tracheotomy care. Oral acetylcysteine may also be used as a mucolytic in less serious cases. Acetylcysteine is the drug of choice for the treatment of paracetamol toxicity. In human medicine, it is also used for the prevention of radiocontrast-induced nephropathy, a form of acute renal failure.

Dose

As mucolytic agent

Horses	:	2 g (total dose), PO, 2 times daily.
Dogs & cats	:	50 ml/hour of 10% or 20% sodium acetylcysteine solution for 30-60 minutes by nebulisation, 2 times daily.

Note: In paracetamol toxicity, acetylcysteine can be given at the dose rate of 150 mg/kg, PO or IV (as 5% solution) initially, followed by 7 mg/kg, PO every 6 hours for 6-7 treatments.

Carbocysteine

Carbocysteine (Carbocisteine) is another derivative of L-cysteine and a mucolytic agent, which liquefies viscid sputum in the same way as acetylcysteine. It is administered by nebulisation or by oral route as syrup. Some patients of chronic bronchitis have been shown to benefit. Its main side effects are GI irritation and rashes. Carbocysteine is contraindicated in peptic ulcers due to mucolytic activity and in the first trimester of pregnancy. It should not be used with antitussives or drugs which dry up bronchial secretions.

Erdosteine

Erdosteine is a thiol derivative mucolytic agent developed primarily for the treatment of chronic obstructive bronchitis. Erdosteine modulates mucus production and viscosity and increases mucociliary transport, thereby improving expectoration. It also exhibits inhibitory activity against the effects of free radicals. In human beings, erdosteine releases three active metabolites in body, which are considered to be mainly responsible for mucolytic and free radical scavenging activity. Erdosteine is associated with low incidence of adverse events, most of which are gastrointestinal and generally mild.

Mucolytic enzymes

Several types of enzymes have been administered by inhalation or instillation into the bronchi to dissolve components of mucopurulent bronchial secretions. These include deoxyribonuclease (DNase), trypsin, streptokinase and streptodornase. Dornase alfa, a highly purified solution of recombinant human deoxyribonuclease I (rhDNase) produced in Chinese hamster ovary cells, selectively cleaves the DNA present in sputum/mucus and reduces viscosity in the lungs, promoting improved clearance of secretions. Side effects of these mucolytic enzymes including airway irritation are not common.

III. DILUENT EXPECTORANTS

Diluent expectorants are hydrating agents which dilute airway mucus after aerosol or systemic administration. Dehydration is common in animals with respiratory diseases because of diminished water intake and excessive water loss associated with pyrexia and hyperpnoea. Thus, rehydration is essential to evacuate airway secretions.

Water aerosols/Steam

Water aerosol or steam is used occasionally to liquefy hyperviscous mucus in the respiratory tract. Although water aerosol may reduce viscosity of respiratory secretions in the large airways, it is unlikely to alter mucus in the smaller bronchi and bronchioles as water droplets more than 10 µm in diameter fail to reach there. In practice, aerosol or mist therapy only delivers a few millilitres of water to the smaller pulmonary airways and lungs, so the steam is not suitable for lung diseases. Further in animals, it is not easier to administer steam. Whenever administered, small animals are simply exposed to steam for repeated short periods or an aerosol of water generated by pumps is fed into a facemask or a small-enclosed cage. Home aerosolisation can be achieved easily with humidifier or steamed bathroom.

Surface acting agents

Several surface-active agents are used occasionally to facilitate hydration, emulsification and liquefaction of thick tenacious bronchial secretions. These include propylene glycol (2-5%), sodium bicarbonate (2-5%) and glycerine (5%). These agents may be administered by nebulisation or instillation at lower concentration directly into the respiratory tract. The efficacy

of surface acting agents compared to water aerosol on liquefaction and enhanced mucus mobility is controversial.

Rehydrating fluids

Administration of parenteral fluids (e.g. normal saline solution), saline aerosols (nebulisation) and oral rehydration fluids are used occasionally to rehydrate the dehydrated animal. The efficacy of these agents, however, remains doubtful.

IV. MISCELLANEOUS EXPECTORANTS

Carbon dioxide

Carbon dioxide gas has been recommended as an efficient expectorant in patients with a thick tenacious secretion in the lower part of respiratory tract. It results in hyperaemia of the bronchial mucosa, which results in less viscous secretion. Carbon dioxide also neutralises anti-expectorant and anti-surfactant action of oxygen in the respiratory tract. Carbon dioxide is administered in a concentration of 5% in small and large animals.

Antimony potassium tartrate

Antimony potassium tartrate (Tartar emetic) is a derivative of tartaric acid. It increases secretions of the respiratory tract by producing nausea. Antimony potassium tartrate is toxic and may cause salivation, cough, metallic taste, nausea, vomiting, bloody diarrhoea, irritability and muscular pains. In higher doses, it causes cardiac arrhythmias leading to cardiac arrest. Skin contact may cause irritation with redness and pain. Use of antimony potassium tartrate as expectorant in animals has declined significantly due to its toxicity and availability of better drugs.

Syrup

Syrup is a thick, viscous liquid consisting primarily of a solution of sugar in water. The concentration of sugar in medicated syrup is generally between 65% and 67% in weight. Syrup has very low expectorant value and is used generally as vehicle for expectorant. On oral administration, it produces soothing demulcent action on the mucosa.

ANTITUSSIVES

Antitussives are agents which are used to relieve or suppress coughing. They are widely used in human practice and are occasionally used in animals in the management of cough. While antitussives may be beneficial in suppressing coughing and in decreasing morbidity in a respiratory disease, they generally do not correct the underlying disorder. Treatment of primary cause is utmost important to get complete relief from coughing.

Pathophysiology of Cough

Cough (Latin: *tussis*) is a sudden and often repetitively occurring reflex that originates due to mechanical or chemical irritation of the pharynx, larynx, tracheobronchial tree and some of small airways. It is a most common symptom associated with a respiratory disease and its main purpose is to remove the foreign material or excess of secretions from the respiratory tract. Although a number of factors may be responsible for triggering coughing, bronchoconstriction is considered as the primary stimulus. Release of humoral agents such as histamine, serotonin or other bronchoconstrictors may be involved in the mechanism.

The cough reflex has a complex pathway. The chemo- and mechano- cough receptors located in the entire tracheobronchial tree or stretch receptors in the lungs respond to cough stimuli (chemical or mechanical) and transmit impulses through afferent nerves such as vagus and glossopharyngeal to the cough centre located in medulla oblongata. The cough centre has coordinating network with respiratory and vomiting centres. The cough centre through efferent pathway supplies nerves to the abdomen, thoracic and diaphragmic muscles and causes forceful expulsion of air from the lungs. Coughing can happen voluntarily as well as involuntarily. Frequent coughing usually indicates the presence of a disease.

Types of cough: Cough can be classified in different ways depending on its duration, character and quality. The duration can be acute (of sudden onset) if it persists for less than three weeks, subacute if it is present between three and eight weeks, and chronic when lasting longer than eight weeks. A cough can be dry or productive, depending on whether sputum is coughed up. The dry and unproductive (non-associated with sputum) cough is usually seen in allergies and in initial stages of bronchitis, whereas the productive cough (associated with large amount of sputum) occurs in later stages of bronchitis and pneumonia.

In the management of cough, it is extremely important to determine the underlying cause and type of cough. Antitussives are indicated only when coughing is painful, unproductive, distressing, exhausting or likely to exacerbate lung damage. They should not be used for productive cough since this would allow collection of fluids and exudates in the bronchial tree. Antitussives should not be employed symptomatically in the absence of diagnosis since indiscriminate inhibition of coughing reflex could have harmful consequences. Further, while antitussives may be beneficial in suppressing coughing so that the patient can get physical and mental rest, they generally do not correct the underlying disorder. Treatment of primary cause is utmost important to get complete relief from coughing.

Classification

Antitussives act by interfering with the cough reflex either at the sensory receptors in pharynx or larynx or by inhibition of the cough centre in medulla. Accordingly, these drugs have been divided into two major groups - peripherally acting and centrally acting antitussives.

I. Peripherally acting/Locally acting antitussives
   1. Demulcents
      e.g. honey, syrup, glycerine and liquorice.
   2. Mucosal anaesthetics
      e.g. benzonatate.
   3. Bronchodilators
      e.g. ephedrine, theophylline and isoprenaline.
   4. Expectorants
      e.g. bromhexine and water aerosols.
   5. Miscellaneous
      e.g. dropropizine and levodropropizine.

II. Centrally acting antitussives
   1. Opioid/Narcotic antitussives
      e.g. codeine, hydrocodone, butorphanol, hydromorphone, dihydrocodeine, acetyldihydrocodeine and morphine.

2. Non-opioid/Non-narcotic antitussives
   e.g. dextromethorphan, noscapine, pholcodine and levopropoxyphene.

I. PERIPHERALLY ACTING/LOCALLY ACTING ANTITUSSIVES

Peripherally acting antitussives are drugs which function only peripherally and relieve coughing by suppressing irritation at the sensory receptors in the pharynx or larynx. These agents include a variety of substances with different pharmacological actions.

1. Demulcents

A number of agents possessing demulcent action including honey, syrup, glycerine and liquorice are often included in various cough remedies. The soothing demulcent action of these agents is effective upon contact and may alley mild irritation of the pharynx for a brief period. They coat, protect and soothe throat directly as well as by promoting salivation and thus reduce afferent impulses originating from irritated/inflamed mucosa. They provide only symptomatic relief in dry cough.

2. Mucosal Anaesthetics

Benzonatate

Benzonatate is a long-chain polyglycol derivative local anaesthetic that is chemically related to other ester local anaesthetics such as procaine and tetracaine. It has more specific actions on mucous membranes and is occasionally used as antitussive. It depresses afferent sensory impulses of the cough reflex and desensitises pulmonary stretch receptors due to its local anaesthetic action. It is also suggested to have some central effects.

Benzonatate is employed clinically to reduce coughing in various respiratory conditions such as bronchitis, emphysema, influenza and pneumonia. It should never be used to suppress a productive cough or in place of treatment of the actual cause of cough, such as asthma treatment. Reported side effects with overdosage of benzonatate include drowsiness, numbness of mouth and throat, and dysphagia. Very high doses can result in overt CNS side effects such as hallucination, restlessness and tremors, followed in extreme cases by convulsions and death.

3. Bronchodilators

Stimulation of pulmonary receptors can induce both cough and bronchoconstriction, especially in patients with bronchial hyperactivity. As bronchospasm generally induces or aggravates coughing, the use of bronchodilators in such cases relieve cough and increase effective volume of respiratory tract. Bronchodilation also improves the effectiveness of cough in clearing secretions. However, bronchodilators should only be used when as element of bronchoconstriction is present and not routinely. Ephedrine, theophylline and isoprenaline are common ingredients employed in some cough preparations. The pharmacology of bronchodilators is given in detail in Chapter 46.

4. Expectorants

Expectorants facilitate removal of the secretions from the respiratory tree and thereby decrease one of the most potent stimulants for coughing. Thus they are believed to 'loosen' cough, which becomes less tiring and more productive. Important agents include mucolytics (e.g. bromhexine) and hydrating (e.g. water aerosols) expectorants. Expectorants are discussed earlier in the present Chapter.

5. Miscellaneous Agents

Dropropizine and Levodropropizine

Dropropizine (Dipropizine) and levodropropizine (l-isomer of dropropizine) are two peripherally acting cough suppressants. Their exact mechanism of anti-tussive action is not known. As their action is limited to periphery, they do not cause central side effects such as sedation or respiratory depression commonly produced by opioid antitussives. The therapeutic use of dropropizine and levodropropizine in veterinary practice appears to be limited.

II. CENTRALLY ACTING ANTITUSSIVES

1. Opioid/Narcotic Antitussives

Opiates and several of their derivatives are potent inhibitors of the medullary cough centre at sub-analgesic doses and can be used specifically as antitussives. However, these agents can be associated with other opioid effects such as sedation, constipation, depression of respiratory centre and dependence, when administered chronically.

Codeine

Codeine (Methyl morphine) is a naturally occurring opiate alkaloid (also prepared semi-synthetically from morphine) and has been a major antitussive for many years. Codeine is available both as the base and the phosphate and sulphate salts. Codeine base is slightly soluble in water, whereas its sulphate and phosphate salts are soluble in water.

Pharmacological effects

Codeine possesses antitussive activity similar to other opiate agonists. It mediates antitussive effect by direct suppression of the cough centre in medulla. It appears to be nearly as effective in depressing the cough as morphine, yet is less depressant on other parts of the CNS. Its analgesic, respiratory depressant and constipating actions are lesser than morphine (see also Chapter 24). Its antitussive action is blocked by naloxone indicating that it is exerted through opioid receptors in the brain. After oral administration, onset of action is usually seen within 30 minutes and antitussive effect persists for 4 – 6 hours.

Pharmacokinetics

Codeine is considered a prodrug, since it is metabolised *in vivo* to the primary active compounds morphine (5-10%) and codeine-6-glucuronide (~70%). It is also converted to norcodeine (~10%) and hydromorphone (~1%). The conversion of codeine to morphine occurs in the liver and is catalysed by the cytochrome P450 enzyme CYP2D6. The active metabolites of codeine, notably morphine, exert their effects by binding to and activating the μ-opioid receptor.

Side effects/Adverse effects

Codeine is generally well tolerated in normal doses. In high doses or with repeated use, it may produce sedation. Potential GI effects of codeine include anorexia, vomiting, constipation, ileus and biliary and pancreatic duct spasms. In very high doses, it may produce respiratory depression and/or CNS depression.

Treatment: Naloxone is the agent of choice in treating respiratory depression induced by codeine. Mechanical respiratory support should also be considered, if respiratory depression is severe.

Contraindications and precautions

Codeine is contraindicated in asthmatics and in patients with diminished respiratory reserves. Codeine should not be given during aerosol therapy, in wet cough or along with mucolytic expectorants as it will depress the cough centre and allow the respiratory secretions to get accumulated in respiratory tract.

Drug interactions

Simultaneous use of other CNS depressants (e.g. anaesthetics, antihistamines, phenothiazines and barbiturates) may increase CNS or respiratory depressant action of codeine. Anticholinergics used with codeine may increase chances of constipation.

Clinical uses

Codeine is indicated mainly to depress an irritating cough that has become chronic and extremely troublesome. In small animal medicine, codeine is used as an oral analgesic and also an antidiarrhoeal (see for detail Chapters 24 and 43).

Dose

For non-productive cough

Dogs : 0.5 – 2 mg/kg, PO, 2 to 3 times daily.
: 10 – 15 mg (total), SC.

Hydrocodone

Hydrocodone (Dihydrocodeinone) is a phenanthrene derivative opiate agonist that occurs as fine white crystals or crystalline powder. It is chemically similar to codeine and has pharmacological activity intermediate to that of codeine and morphine. Like codeine, it mediates antitussive effect by direct suppression of the cough centre in medulla and tends to have slightly greater antitussive effect than codeine. Hydrocodone may also be more sedating than codeine but its respiratory depressant and constipating effects are less than codeine.

Side effects of hydrocodone are usually related to codeine and may include sedation, constipation (with chronic therapy), vomiting or other GI disturbances. In very high doses, it may cause CNS, cardiovascular and respiratory depression secondary to the opiate effects. Drug interactions and contraindications of hydrocodone are similar to those of codeine. Hydrocodone is recommended in treatment of harsh, dry and non-productive cough associated with bronchitis and bronchiectasis in dogs. The pharmacology of hydrocodone has also been given in Chapter 24.

Dose

For non-productive and irritating cough

Dogs : 0.25 mg/kg, PO, 2 to 4 times daily.

Butorphanol

Butorphanol tartrate is a synthetic opiate partial agonist (agonist/antagonist). It is structurally related to morphine but exhibits pharmacological actions similar to other partial agonists such as pentazocine or nalbuphine (see for detail Chapter 24). It is a potent analgesic and antitussive agent. After oral or IM administration, butorphanol is almost completely absorbed but undergoes a significant first-pass metabolism after oral absorption. The detailed pharmacology of butorphanol has been discussed in Chapter 24.

Butorphanol is recommended for the relief of chronic non-productive cough associated with tracheobronchitis, tracheitis, tonsillitis, laryngitis and pharyngitis originating from inflammatory conditions of the upper respiratory tract in dogs. Its other uses in dogs and cats are as pre-anaesthetic and analgesic and as an antiemetic prior to cisplatin treatment. In horses, it is indicated for the relief of pain associated with colic. Adverse effects reported in dogs include sedation (occasionally), and anorexia and diarrhoea (rarely). Other effects like ataxia, respiratory depression and salivation may be seen following very high doses. Other CNS depressant drugs may cause increased CNS depression when used with butorphanol; in such cases dosage may need to be decreased.

Dose

For non-productive cough

Dogs : 0.1 – 0.25 mg/kg, IM or SC, 2 to 3 times daily for 7-14 days.
: 0.5 mg/kg, PO, 2 to 3 times daily for 7-14 days.
Cats : 0.05 – 01 mg/kg, SC, 2 to 3 times daily.

Hydromorphone

Hydromorphone (Dihydromorphinone) HCl, a morphine derivative, is a powerful analgesic. Its antitussive properties are similar to codeine, but it tends to strongly depress the respiratory mechanisms. Hydromorphone is used in medicine as an alternative to morphine for analgesia, and as a second- or third-line narcotic antitussive for cases of dry, painful, paroxysmal coughing resulting from continuing bronchial irritation after viral infection, inhalation of fungus, and other causes. Adverse effects of hydromorphone are similar to those of other opioid analgesics, such as morphine. It does not appear to have significant advantage over codeine and is less used in veterinary practice.

Dihydrocodeine

Dihydrocodeine (DHC) is a semi-synthetic opioid derivative and a close congener of codeine. Similar to codeine, dihydrocodeine possesses significant cough suppressant action and also analgesic activities. The antitussive dose of DHC, however, tends to be lesser than the analgesic dose. The side/adverse effect profile and drug interactions are similar to those of codeine. As with other opioids, tolerance, and physical and psychological dependence develop with repeated use of dihydrocodeine. Dihydrocodeine is considered the parent drug of a series of other moderately strong opioid antitussives including hydrocodone, nicocodeine, nicodicodeine and acetyldihydrocodeine.

Acetyldihydrocodeine

Acetyldihydrocodeine is an opiate derivative developed as a cough suppressant and analgesic. It is chemically a 6-acetyl derivative of dihydrocodeine. It has higher lipophilicity than codeine and is converted *in vivo* to dihydromorphine rather than morphine. It has activity similar to other opiates, but is not commonly used. It can be expected to be more potent and longer lasting, and also have higher bioavailability than codeine. Side effects are similar to those of other opiates and include itching, nausea and respiratory depression.

Other agents

Several natural, semi-synthetic and synthetic opioids possess antitussive properties and can be used occasionally for this purpose. These include natural and semi-synthetic opiates morphine, camphorated tincture of opium (paregoric), ethyl morphine, benzylmorphine, dihydroisocodeine, nicocodeine, nicodicodeine and oxycodone. Among synthetic agents are

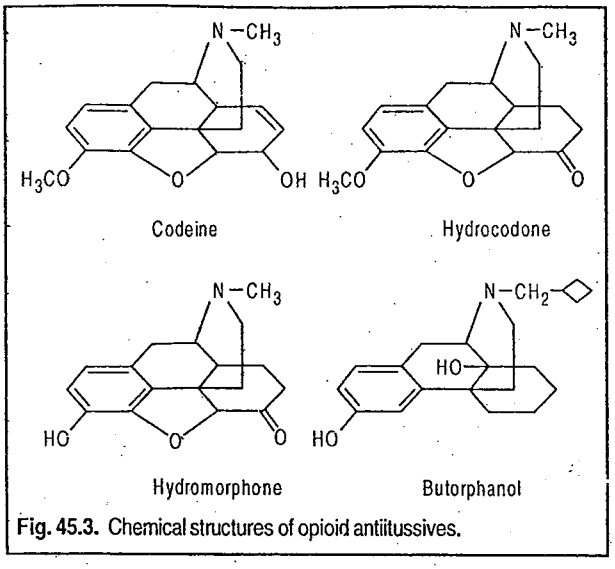


Fig. 45.3. Chemical structures of opioid antiitussives.

tipepidine, levomethadone and normethadone. Morphine has good antitussive activity at sub-analgesic doses, but is seldom used for this purpose because of high abuse liability in human beings.

2. Non-Opioid/Non-Narcotic Antitussives

Non-opioid (non-narcotic) antitussives have been developed to increase the safety of centrally acting antitussives. These drugs retain the ability of codeine to suppress the cough centre but have very limited other CNS effects like analgesia, respiratory depression and addictive properties.

Dextromethorphan

Dextromethorphan (d-3-Methoxy-N-methylmorphinan) is the d-isomer of codeine analogue levorphanol. As the pure free base, dextromethorphan occurs as an odourless, white to slightly yellow crystalline powder. It is commonly available as the monohydrated hydrobromide salt, however some newer extended-release formulations contain dextromethorphan bound to an ion exchange resin based on polystyrene sulfonic acid. Dextromethorphan is used primarily as an antitussive agent in both human and veterinary practices.

Mechanism of action

Dextromethorphan has been reported to possess multiple actions in body, but its exact mechanism of anti-tussive effect is not clearly known. It acts as an uncompetitive NMDA receptor (PCP site) antagonist. It also acts as σ_1 and σ_2 sigma receptor agonist, and serotonin and norepinephrine transporters (SERT and NET) blocker at different concentrations. However, unlike codeine, its antitussive action is not blocked by naloxone, which indicates that its central antitussive effect is mediated by a non-opioid mechanism(s).

Pharmacological effects

Dextromethorphan possesses selective antitussive action and acts centrally to raise threshold of cough centre. Unlike the l-isomer, it has no analgesic or addictive properties and does not act through opioid receptors. Although it is as effective as codeine, dextromethorphan does not depress mucociliary function of the airways mucosa and is practically devoid of constipating and other GI side effects after oral administration.

Pharmacokinetics

Following oral administration, dextromethorphan is rapidly absorbed from the gastrointestinal tract, where it enters bloodstream and crosses the blood-brain barrier. It has a rapid onset of action with full effect attained in about 30 minutes in dogs. In the liver it is metabolised by cytochrome P-450 enzyme system to an active metabolite dextrorphan. The therapeutic activity of dextromethorphan is believed to be caused by both the drug and the active metabolite. The duration of action in human patients after oral administration is approximately three to eight hours for dextromethorphan-hydrobromide, and ten to twelve hours for dextromethorphan-polistirex.

Side effects/Adverse effects

Compared with codeine, dextromethorphan has fewer CNS and GI side effects. Its toxicity is low with occasional nausea and dizziness, but extremely high doses may produce CNS depression. In human beings, dextromethorphan has been associated with sudden histamine release in some children. In general, it is accepted as a safe and effective antitussive agent and is among the most extensively used components of cough preparations.

Contraindications and precautions

Dextromethorphan should not be used in patients susceptible to allergic reactions due to its susceptibility to cause sudden histamine release.

Drug interactions

Dextromethorphan should not be administered along with monoamine oxidase inhibitors (MAOIs) or serotonin reuptake inhibitors (like SSRIs, and TCAs) due to the potential for serotonin syndrome, a potentially life-threatening condition.

Clinical uses

Dextromethorphan is recommended mainly for non-productive cough. Though it is a synthetic morphine derivative, yet dextromethorphan can safely be used in cats. Studies in humans have shown that combination of dextromethorphan with a bronchodilator is superior to dextromethorphan alone.

Dose

Dogs & cats : 1 – 2 mg/kg, PO, 3 to 4 times daily.

Noscapine

Noscapine (Narcotine, anoscapine, anarcotine) is an opium alkaloid of the benzoisoquinoline series. It exists in opium as a free base (1.3 to 10 percent) but possesses no narcotic properties. To eliminate misunderstanding of being a narcotic, the name noscapine is employed in the pharmaceutical literature, although narcotine still remains the common chemical designation of this alkaloid. Its antitussive effects appear to be primarily mediated by its sigma receptor agonist activity because pretreatment with rimcazole, a sigma specific antagonist, causes a dose-dependent reduction in antitussive activity of noscapine.

Noscapine has a potent antitussive action similar to dextromethorphan, almost equalling that of codeine. Except for its antitussive effect, it has no significant effect on CNS in doses within therapeutic range. However, the drug is a potent releaser of histamine in dogs and its large doses cause bronchoconstriction and transient hypotension. Some of the side effects reported in humans are drowsiness, headache, nausea and allergic rhinitis. Noscapine should not be taken with any monoamine oxidase inhibitors (MAOIs), as unknown and potentially fatal effects may occur. Noscapine has also shown to significantly increase the

anticoagulant effects of warfarin and is therefore not used in conjunction with warfarin. The clinical use of noscapine in small animals appears to be limited.

Pholcodine

Pholcodine, also known as morpholinylethylmorphine, is structurally related to opioids, but has no analgesic or addicting properties. It is about twice as potent as codeine. Pholcodine readily absorbs from the gastrointestinal tract and freely crosses the blood-brain barrier. It has a long half-life and can be given once or twice daily. It is used mainly as antitussive to suppress unproductive cough. It also possesses an antifungal action. Pholcodine is well tolerated with limited side effects of gastrointestinal disturbances such as nausea or vomiting.

Levopropoxyphene

Levopropoxyphene napsylate is the l-isomer of dextropropoxyphene. Unlike dextropropoxyphene, it has little or on analgesic activity. Its antitussive potency is about 1/3 to 1/2 to that of dextromethorphan.

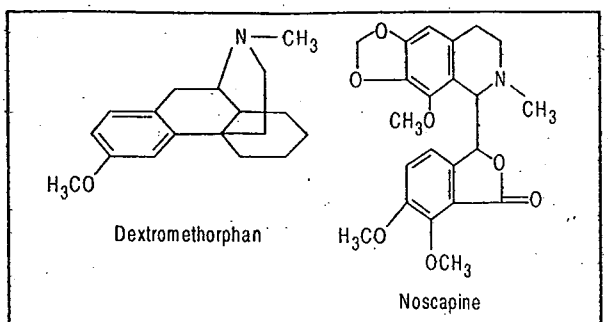


Fig. 45.4. Chemical structures of selected non-opioid antitussives.

Other drugs

Several other drugs belonging to different pharmacological classes have been used occasionally as centrally acting antitussives. These include butamirate, carbetapentane, caramiphen, clofedanol (chlophedianol), dimemorfan, glaucine, oxeladin, pipazethate and zipeprol. Many first generation H_1-receptor antagonists (e.g. diphenhydramine, chlorphenamine and promethazine) are conventionally added to antitussive formulations to provide relief from cough in respiratory allergic conditions.

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CHAPTER 46 BRONCHODILATORS AND DRUGS USED FOR ALLERGIC AND INFLAMMATORY DISORDERS OF RESPIRATORY TRACT

Bronchodilators
- Pathophysiology of bronchoconstriction
- Classification
- Adrenergic agonists/Sympathomimetics
  - Mechanism of action
  - Non-selective beta-adrenergic receptor agonists
  - Selective $beta_2$-adrenergic receptor agonists
- Muscarinic receptor antagonists
- Methylxanthines/Xanthines
  - Chemistry and structure-activity relationships
  - Mechanisms of action
- Leukotriene antagonists

Drugs used for allergic and inflammatory disorders of respiratory tract/Asthma
- Pathophysiology of asthma

Drugs used for allergic and inflammatory disorders of respiratory tract/Asthma
- Classification
- Anti-inflammatory drugs
  - Corticosteroids
  - Non-steroidal anti-inflammatory drugs
- Bronchodilators
- Mast cell stabilisers
- Antihistamines
- Miscellaneous drugs
  - Serotonin antagonists
  - Decongestants

BRONCHODILATORS

Bronchodilators are drugs which cause an increase in calibre of bronchus and bronchial tube. They are used where there is suspicion of bronchial narrowing due to bronchial secretion or bronchoconstriction, or where improved alveolar ventilation is required. They are most useful in obstructive lung diseases, of which asthma and chronic obstructive pulmonary disease are the most common conditions.

Pathophysiology of Bronchoconstriction

The tone of bronchial muscle is controlled by humoral factors and autonomic nervous system. The nervous innervations to the smooth muscle of the respiratory tract are complex, but the overall balance is maintained by both parasympathetic and sympathetic nervous systems. The parasympathetic nervous system provides primary innervations responsible for baseline tone of mild bronchoconstriction that characterises the normal respiratory tract. The sympathetic system balances these effects by stimulating $beta_2$-adrenoceptors to induce bronchodilation. A third not well characterised nervous system, the nonadrenergic-noncholinergic (NANC) system (e.g. vasoactive intestinal peptide) also innervates bronchial smooth muscle and mediates bronchodilation.

The intracellular mechanisms which transmit signals from the nervous system to smooth muscle depend in part on changes in the intracellular concentration of cyclic AMP and cyclic GMP. The cyclic AMP is decreased by alpha-adrenergic stimulation and increased by $beta_2$-adrnoeceptor stimulation. Stimulation of muscarinic (cholinergic) and histaminergic receptors cause mobilisation of intracellular Ca^{++}, which in turn results in contraction of smooth muscle through a variety of Ca^{++} mediated effects. Smooth muscle receptors are also susceptible to stimulation by a variety of chemical mediators and drugs, which may modulate cAMP and cGMP concentrations.

In veterinary medicine, bronchodilators are used for respiratory disorders including tracheobronchitis and chronic pulmonary disease in horses, chronic bronchitis in dogs, asthma syndrome in cats, and bronchopneumonia and chronic interstitial disease in all species. Bronchoconstriction produced by chemical mediators in hypersensitivity and inflammatory conditions often requires use of bronchodilators with or without concurrent use of other drugs (e.g. corticosteroids and antihistamines). The choice and use of bronchodilators may be primarily on an empirical basis. The assessment of airway function, airway calibre and bronchomotor tone is often subjective.

Classification

I. Adrenergic agonists/Sympathomimetics
   1. Non-selective beta-adrenergic receptor agonists.
      e.g. epinephrine, ephedrine, isoprenaline and orciprinaline.
   2. Selective $beta_2$-adrenergic receptor agonists
      - e.g. clenbuterol, terbutaline, salbutamol and salmeterol.

II. Muscarinic receptor antagonists/Anticholinergics
   e.g. atropine, glycopyrronium, ipratropium, tiotropine and eucatropine.

III. Methylxanthines
   e.g. theophylline, aminophylline and etamiphylline.

IV. Leukotriene antagonists
   e.g. montelukast and zafirlukast.

I. Adrenergic Agonists/Sympathomimetics

Adrenergic agonists cause bronchodilation through activation of beta$_2$-adrenoceptor present in respiratory tract. Beta-receptor agonists are considered the most effective group of bronchodilators because they act as functional antagonist of airway constriction, regardless of the type of stimuli.

Mechanism of Action

Adrenergic agonists interact with beta$_2$-adrenergic receptors located on several cell types in respiratory tract, including the smooth muscle and the inflammatory cells. The interaction between β_2-agonist and receptor causes conformational changes in the receptor and subsequent activation of adenylate cyclase on the inner cell membrane, which converts ATP to cAMP (Figure 46.1). Cyclic AMP in turn serves as a second messenger for the activation of specific kinases. The kinases activate certain enzymes which cause relaxation of airway smooth muscle. In the inflammatory cells, increased cAMP concentration inhibits antigen-antibody reaction induced by release of mediators like histamine. Beta-adrenergic receptors also stimulate secretion of airway mucus, resulting in a less viscous secretion and enhanced ciliary activity.

Adrenergic agonists are mostly administered by inhalation of aerosol, powder or nebulised solution in human beings, but some may be given also orally or by injection. Adrenergic agonists are regarded as the fastest acting bronchodilators when inhaled. Adrenergic drugs are the mainstay of treatment of reversible airway obstruction, but should be cautiously used in hypersensitive heart patients and those receiving digitalis.

1. Non-Selective Beta-Adrenergic Receptor Agonists

Non-selective beta-adrenergic receptor agonists are an important component of the therapy of acute respiratory distress in dogs and cats. These drugs, however, may cause adverse cardiac effects as a result of beta$_1$-receptor stimulation.

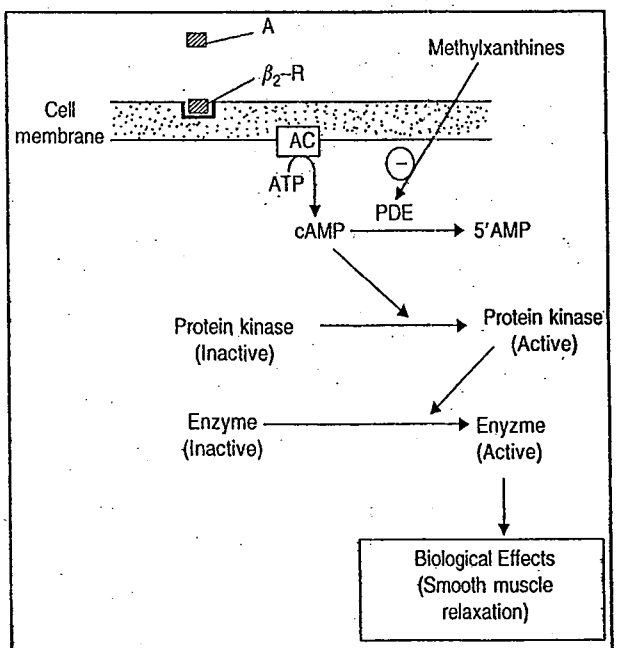


Fig. 46.1. Schematic representation of mechanism of action of beta-adrenoceptor agonists (see text for detail). **Abbreviations:** A = adrenoceptor agonist; β_2- R = beta$_2$ - adrenoceptor ; ATP = adenosine triphosphate ; cAMP = 3', 5'-cyclic adenosine monophosphate; 5' AMP = adenosine 5' - monophosphate ; PDE = phosphodiesterase enzyme ; – = inhibition.

Epinephrine

Epinephrine (Adrenaline) acts on both alpha and beta (β_1 and β_2) receptors and is a potent bronchodilator. It produces prompt but short lasting bronchodilation. In addition to beta$_2$-receptor stimulant activity, epinephrine has considerable alpha-adrenergic activity which may cause vasoconstriction and systemic hypertension. It also possesses alpha action on bronchi (weakly bronchoconstriction), but it is masked by the dominant beta$_2$ mediated relaxation. Though the clinical use of epinephrine as a bronchodilator has declined in recent years due to availability of selective drugs, yet it is considered as the drug of choice for parenteral injection (SC, IM or even IV) in severe anaphylaxis to achieve rapid effect. The pharmacology, clinical uses and doses of epinephrine are given in Chapter 11.

Ephedrine

Ephedrine is a mixed acting non-catecholamine, which stimulates both alpha- and beta-receptors and CNS also. It causes slowly developing mild bronchodilation. Ephedrine is used mainly as a chronic medication for mild to moderate asthma. As it also possesses alpha-agonistic properties, ephedrine produces decongestion in the bronchi which helps to reduce mucosal swelling. However, like epinephrine, it may cause systemic vasoconstriction and hypertension which are not desirable. The pharmacology of ephedrine has been described in Chapter 11.

Isoprenaline

Isoprenaline (Isoproterenol) is a non-selective β_1 and β_2-adrenoceptor agonist with no activity on alpha-adrenergic receptors. It has an advantage that it can be used both parenterally to achieve rapid effect and orally for chronic therapy. It causes prompt and marked bronchodilation lasting for 1-2 hours. Like other non-selective beta-adrenoceptor agonists, isoprenaline may cause adverse effects as a result of beta$_1$-adrenergic receptor stimulation. These effects may be problematic in cats suffering from hypertrophic cardiomyopathy. Aerosolisation reduces the adverse effects of isoprenaline by increasing beta$_2$-receptor specificity, but aerosol inhalation is not a convenient means of administration of drugs for animals. The pharmacological effects, adverse effects and doses of isoprenaline have been given in Chapter 11.

Orciprinaline

Orciprinaline (Metaproterenol) is a long-acting derivative of isoprenaline. Its activity resembles isoprenaline (β_1 and β_2 adrenoceptor agonistic action), but is somewhat more selective β_2-receptor agonist with less cardiac stimulation. It is not metabolised by COMT, so after oral administration orciprinaline shows longer effect than isoprenaline. When inhaled as an aerosol, it acts promptly and the desirable action lasts for 3-6 hours.

2. Selective Beta$_2$-Adrenergic Receptor Agonists

Selective beta$_2$-adrenoceptor agonists act selectively on β_2-receptors in the respiratory tract smooth muscles and are not generally associated with the undesirable effects of beta$_1$-adrenergic stimulation. Presently, these drugs are more commonly used for bronchodilation than non-selective adrenoceptor agonists.

Clenbuterol

Clenbuterol HCl is a direct acting sympathomimetic, which acts selectively on β_2-receptors. It was introduced in veterinary

medicine for the relief of bronchospasm in horses. It is also available for use in human patients as a decongestant and bronchodilator. Clenbuterol is a long-acting $beta_2$-receptor agonist. In addition to its bronchodilator action, clenbuterol increases bronchial secretions and improves the clearance of bronchial contents by ciliary action.

Clenbuterol is approved for horses as a bronchodilator in the management of airway obstruction such as chronic obstructive pulmonary disease. It is also useful in other species in allergic respiratory diseases, respiratory infection and inflammation. Clenbuterol has also been used as a performance-enhancing drug. Its ability to increase the muscle-to-fat body ratio makes its illegal use in livestock popular to obtain leaner meats. The pharmacology, clinical uses and doses of clenbuterol are given in Chapter 11.

Terbutaline

Terbutaline sulphate is a synthetic sympathomimetic amine, which stimulates selectively $beta_2$ adrenergic receptors found mainly in bronchial, vascular and uterine smooth muscles causing their relaxation. Similar to clenbuterol, it increases ciliary beating of respiratory mucosal cells and has a mucolytic action, which may contribute to its therapeutic effect. Terbutaline may have a greater cardiac stimulant effect than clenbuterol, which may be more pronounced in cats. Rapid first-pass metabolism of terbutaline results in reduced systemic bioavailability following oral administration. Terbutaline is used as bronchodilator mainly in the adjunctive treatment of cardiopulmonary diseases like tracheobronchitis, collapsing trachea, allergic bronchitis and pulmonary oedema in small animals and horses. The pharmacology, clinical uses and doses of terbutaline are given in Chapter 11.

Salbutamol

Salbutamol (Albuterol) is a short-acting selective $beta_2$-adrenoceptor agonist. Like clenbuterol and terbutaline, it possesses bronchodilator activity but has minimal cardiac stimulant action. It is used principally in dogs and cats for its effects on bronchial smooth muscle to alleviate bronchospasm or cough. It could also be used in horses as a bronchodilator. Its action, adverse effects, and clinical uses are like other $beta_2$-adrenoceptor agonists (see for detail Chapter 11).

Salmeterol

Salmeterol is a long-acting $beta_2$-adrenergic receptor agonist drug. It possesses an aryl alkyl group with a chain length of 11-atoms at the amine, which makes the compound more lipophilic and more selective for beta adrenergic receptor providing long duration of action. Salmeterol is currently prescribed for the treatment of asthma and chronic obstructive pulmonary disease (COPD).

Other selective $beta_2$-adrenoceptor agonists

Several other relatively selective β_2-adrenoceptor agonists are available, but do not offer any special advantage over the drugs mentioned above. These include bitolterol, fenoterol, isoetharine, levosalbutamol, pirbuterol and rimiterol. Some of these drugs have been discussed briefly in Chapter 11.

II. Muscarinic Receptor Antagonists

Muscarinic receptor antagonists (Anticholinergics) decrease vagal tone in bronchiolar smooth muscle, and may be useful in certain cases of bronchoconstriction. However, they are less efficacious than adrenergic agonists but can add to their response. Although the therapeutic use of anticholinergics as bronchodilators has declined, the addition of newer quaternary anticholinergics such as ipratropium and tiotropium has renewed interest in this group of drugs. The routes by which anticholinergics are administered also influence their bronchodilatory effects.

Atropine

The antimuscarinic drug atropine has been used for many years for the relief of bronchoconstriction in chronic emphysema in horses. Atropine reduces the muscarinic cholinergic receptor mediated bronchoconstriction to produce relaxation of tracheobronchial smooth muscle. However, it reduces secretions in the respiratory tract with an increase in mucus viscosity, which is not desirable. Additionally, it produces a number of systemic effects like tachycardia, xerostomia, CNS stimulation and altered GI and urinary tract functions due to its non-selective action on the muscarinic receptors. Aerosolisation of atropine does not reduce the incidence of its adverse effects. All these factors limit its routine use as a bronchodilator.

The primary indication of atropine as a bronchodilator in small animals is facilitation of bronchodilation in acute dyspnoeic animal. Combination of atropine with either a beta-adrenoceptor agonist or a glucocorticoid causes better bronchodilation than either drug used alone. The detailed pharmacology of atropine has been given in Chapter 14.

Dose

Dogs & cats : 0.02 – 0.04 mg/kg, IM, SC or IV.

Glycopyrronium

Glycopyrronium (Glycopyrrolate) is a synthetic quaternary ammonium antimuscarinic agent that can be used as a bronchodilator in small animals. It is twice as potent as atropine in causing bronchodilation when aerosolised. Although its onset of action is slower than that of atropine, its half-life is 4 to 6 times compared to 1 to 2 hours of atropine. After parenteral administration it can have a similar adverse effect profile as atropine, with the exception of CNS effects. On aerosolisation, systemic effects of glycopyrronium are minimal (see also Chapter 14).

Dose

Dogs & cats : 0.01 – 0.02 mg/kg, IM, SC or IV.

Ipratropium

Ipratropium bromide is a synthetic quaternary ammonium antimuscarinic compound. It is used as a bronchodilator mainly in human medicine and seldom in horses.

Mechanism of action

Ipratropium is a non-selective muscarinic receptors blocker. The blockade of muscarinic acetylcholine receptors in the smooth muscles results in decreased synthesis of intracellular cyclic guanosine monophosphate (cGMP). It is postulated that reduced concentration of cGMP produces negative effect on intracellular calcium, which results in decreased contractility of smooth muscle in bronchi and lungs, thereby inhibiting bronchoconstriction and mucus secretion.

Pharmacological effects

Ipratropium antagonises muscarinic receptors in the bronchiolar smooth muscles and is considered pharmacologically superior to atropine. Although the two drugs are equipotent and produce similar systemic effects (e.g. bronchodilation, xerostomia and tachycardia) when given parenterally, ipratropium due to its

charged nature does not cross blood-brain barrier and lacks appreciable effect on the CNS. Unlike atropine, it does not alter mucociliary transport rates after both local and parenteral administration. When aerosol is inhaled, the actions of ipratropium are confined almost exclusively to the mouth and airways. Even when administered in amounts many times the recommended dosages, little or no systemic effects are seen. This selectively results from inefficient absorption (<1%) of the drug from the lungs or GI tract. The bronchodilation produced by ipratropium develops more slowly (about 30 minutes) and is usually less intense than that produced by sympathomimetics. In heaves-affected horses, ipratropium bromide nebulisation has shown to cause bronchodilation lasting about 6 hours. Dry powder inhalation of this agent has also been shown to improve pulmonary function of affected horses.

Side effects/Adverse effects

Ipratropium may produce transient local side effects like dryness of mouth, irritation in trachea, cough and nervousness in few patients, but systemic effects are rare.

Contraindications and precautions

There are no contraindications for inhaled ipratropium, apart from hypersensitivity to atropine and related substances. For oral administration, contraindications are similar to other anticholinergics i.e. narrow angle glaucoma and obstructions in the gastrointestinal tract and urinary system.

Drug interactions

Combination with beta-adrenergic agonists, as well as theophylline and other xanthine derivatives with ipratropium, increases the dilating effect on the bronchi.

Clinical uses

The main clinical use of ipratropium in veterinary medicine is in the treatment of chronic obstructive pulmonary disease (COPD). In acute bronchoconstriction, the combination of a beta$_2$-adrenoceptor agonist and ipratropium is considered more effective than either agent alone. Ipratropium is also useful in reducing nasal flow in rhinorrhoea, but will not help in decreasing nasal congestion. Nebulizer solution containing ipratropium bromide 250 micrograms/mL is available; it is diluted to 75 micrograms/ml in sterile sodium chloride for use in horses.

Dose

For bronchodilation (Ipratropium bromide 75 micrograms/ml solution)

Horses : 2–3 micrograms/kg, by inhalation, up to 4 times daily.

Tiotropium

Tiotropium is a newly developed congener of ipratropium bromide that binds very tightly to bronchial muscarinic receptors producing long lasting bronchodilation. Like ipratropium, it is not absorbed from respiratory tract and GI mucosa and exhibits high selectivity for bronchial smooth muscle. Tiotropium is used in the management of chronic obstructive pulmonary disease (COPD).

Other anticholinergic agents

Several other anticholinergic drugs like eucatropine, deptropine and oxitropium can be used as bronchodilators and can augment the bronchodilator effect of adrenergic agonists.

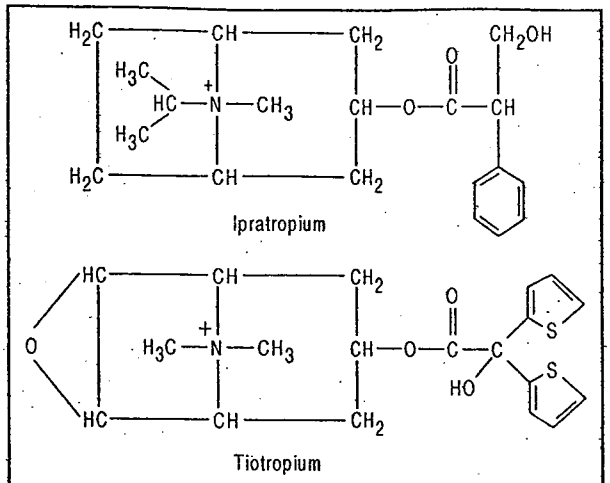


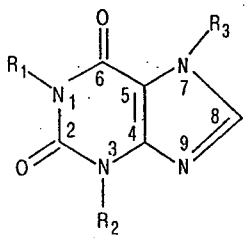
Fig. 46.2. Chemical structures of selected anticholinergic bronchodilators.

III. Methylxanthines/Xanthines

Methylxanthines include three naturally occurring alkaloids caffeine, theophylline and theobromine and their derivatives. The naturally occurring alkaloids are consumed primarily as beverages (e.g. tea, coffee and cocoa) and have CNS stimulant (Chapter 25), diuretic (Chapter 39), myocardial stimulant (Chapter 32) and smooth muscle relaxant properties. All the three alkaloids have qualitatively similar actions, but there are marked quantitative and pharmacokinetic differences. In general order of potency for naturally occurring methylxanthines is theophylline > caffeine > theobromine. Methylxanthines such as theophylline and its compounds have been extensively used as bronchodilators in asthma, but are not considered the first line drugs any more. Traditionally, theophylline has been favoured for smooth muscle relaxation (including bronchodilation) and caffeine for CNS stimulation (including vasomotor centre stimulation). Theobromine is of no therapeutic importance.

Chemistry and Structure-activity relationships

Xanthine (3,7-Dihydro-purine-2,6-dione) is an organic heterocyclic compound. Caffeine, theophylline and theobromine are all methylated xanthines i.e. caffeine is 1, 3, 7-trimethylxanthine,



General Structure

Compound	R_1	R_2	R_3
Xanthine	– H	– H	– H
Caffeine	– CH_3	– CH_3	– CH_3
Theophylline	– CH_3	– CH_3	– H
Theobromine	– H	– CH_3	– CH_3

Fig. 46.3. Chemical structures of xanthine and naturally occuring methylxanthines.

theophylline is 1, 3-dimethylxanthine, and theobromine is 3, 7-dimethylxanthine (Figure 46.4). These natural alkaloids have low solubility, which can be enhanced by formation of complexes with other compounds e.g. between theophylline and ethylenediamine to give aminophylline and between caffeine and sodium benzoate. Substitution at position 3 on xanthine molecule augments the bronchodilator potency, while substitution at position 7 decreases bronchodilator potency (e.g. theophylline is more potent than caffeine or theobromine as bronchodilator). Congeners of theophylline with large non-polar substituents at positions 1 and 3 usually display enhancement of ability to inhibit cyclic nucleotide phosphodiesterase and to antagonise receptor mediated actions of adenosine.

Mechanisms of Action

Three distinct mechanisms of actions for methylxanthines have been identified.

a. Methylxanthines competitively inhibit phosphodiesterase enzyme, which degrades cyclic nucleotides (cAMP and cGMP) intracellularly. The relaxant effect on smooth muscle has been attributed to increased cAMP (also cGMP) levels. The increased levels of cAMP activate protein kinase A (PKA), inhibit synthesis of leukotrienes and tumour necrosis factor-alpha (TNF-α) and suppress release of histamine. This reduces inflammation and innate immunity. Bronchodilation, cardiac stimulation and vasodilatation also occur when cAMP levels rises in the concerned cells. Methylxanthines also release catecholamines from adrenal medulla, which further elevate levels of cAMP in cells.

b. Methylxanthines cause release of Ca^{++} from sarcoplasmic reticulum, especially in the skeletal and cardiac muscles. This uncoupling of intracellular Ca^{++} increases with muscle contractile elements.

c. Methylxanthines produce blockade of adenosine receptor. Adenosine acts as a local mediator in CNS, cardiovascular system and other organs causing contraction of smooth muscles (especially bronchial muscles), dilation of cerebral blood vessels, suppression of cardiac pacemaker and inhibition of gastric secretion. Methylxanthines by antagonising adenosine produce opposite effects. It is now suggested that adenosine receptor antagonism is the most important factor responsible for many pharmacological effects of methylxanthines in doses which are recommended therapeutically. First two actions (a and b) of methylxanthines are exerted only at concentrations much higher than their therapeutic plasma concentrations.

Different xanthine analogues show varying potency at the numerous subtypes, and a wide range of synthetic xanthines (some non-methylated) have been developed searching for compounds with greater selectivity for phosphodiesterase enzyme or adenosine receptor subtypes.

Theophylline

Theophylline (1, 3-Dimethylxanthine) occurs as a bitter tasting, odourless, white crystalline powder. It is sparingly soluble in alcohol and only slightly soluble in water at pH of 7, but solubility increases with increasing pH. It is considered the prototype methylxanthine and has been discussed in detail in the present chapter.

Pharmacological effects

Theophylline exerts multiple pharmacological effects at different body systems.

Respiratory system: Theophylline relaxes all smooth muscles of body, but the most prominent effect is seen on the smaller airways of respiratory tract. It produces bronchodilation by its direct action of respiratory smooth muscle and may also increase tidal volume by stimulating the respiratory centre in medulla oblongata. In addition to its bronchodilatory effect, theophylline inhibits mast cells degranulation and thus histamine release, increases mucociliary clearance and prevents microvascular leakage in the respiratory tract. It is more effective in conditions with reversible airway obstruction than in chronic respiratory diseases. Its ability to induce bronchodilation is severely impaired by pathological changes both in airway walls and pulmonary interstitium. This accounts for the wide variability of response seen with methylxanthines.

Central nervous system: Theophylline and caffeine are potent stimulants of the CNS. Though theophylline induced CNS effects are less potent than caffeine, theophylline is more important for its CNS induced adverse effects. Both methylxanthines produce alertness, allay fatigue, improve performance and increase motor activity. They also stimulate medullary, vagal, respiratory and vasomotor centres. Vomiting at high doses is due to gastric irritation and chemoreceptor trigger zone (CTZ) stimulation.

Cardiovascular system: Theophylline (also caffeine) directly stimulates heart and increases force of myocardial contraction. However, the action of methylxanthines on cardiovascular system are complex and sometimes antagonistic and the resulting effects largely depend on prevailing conditions, doses administered and previous history of methylxanthine exposures. For example, theophylline tends to increase heart rate by direct action, but decreases it by vagal stimulation; the net effect is variable. Similarly, it produces vasodilatation in most blood vessels but produces vasoconstriction in cerebral blood vessels. Effects on blood pressure is also variable and unpredictable as vasomotor tone and direct cardiac stimulation tend to increase blood pressure but vagal stimulation and direct vasodilatation tend to decrease blood pressure.

Kidneys: Theophylline has a mild diuretic effect (more than caffeine). It acts by inhibiting tubular reabsorption of Na^+ and water and by causing increased renal blood flow and glomerular filtration rate. Theophylline is more potent than caffeine, but it has shorter action.

Skeletal muscle: Theophylline by direct action enhances contractile power of skeletal muscle through release of Ca^{++} from sarcoplasmic reticulum. In addition, there is enhanced release of ACh, which facilitates neuromuscular transmission. Central action of these drugs relieves fatigue and increase muscular work. Enhanced diaphragmatic contractility noted with theophylline in the therapeutic concentration range probably contributes to its beneficial effect.

Gastrointestinal tract: Theophylline enhances secretion of acid and pepsin in stomach. It may also relieve biliary spasm, but effect on intestinal smooth muscle in negligible.

Pharmacokinetics

Theophylline is well absorbed after oral administration. Administering the drug after a meal high in fat content may slow

down the absorption process, without affecting the bioavailability. It has serum protein binding of about 10% in small animals. Theophylline is distributed throughout extracellular fluids and body tissues. It crosses placenta and distributes into milk. The volume of distribution may increase in neonates and those suffering from cirrhosis or malnutrition, whereas the volume of distribution may decrease in obese animals. It is metabolised primarily in the liver to 3-methylxanthines, which has weak bronchodilatory activity. The reported elimination half-life of theophylline in various species is: dogs = 5-7 hours, cats = 7-8 hours, pigs = 11 hours and horses = 17 hours.

Side effects/Adverse effects

Theophylline has a low therapeutic index. It is associated with a wide range of adverse effects including CNS excitation (restlessness, tremors and seizures), GI upsets (anorexia, pain, nausea, vomiting and diarrhoea), cardiac stimulation (tachycardia) and diuresis. Rapid infusion of drug has been reported to cause cardiac arrhythmias, hypotension, nausea, tremors and acute respiratory failure. Most adverse effects are related to serum levels of the drug and to a large degree may be avoided by appropriate dosing based on the clinical experience and response of individual animals. Theophylline has low volume of distribution and low lipid solubility; therefore obese patients should be dosed on a lean body weight basis.

It is contradicted in acute myocardial disease. It has toxic interaction with erythromycin.

Clinical uses

Theophylline can be used in respiratory diseases where bronchodilation may be beneficial. Since beta$_2$-adrenoceptor agonists promote the synthesis of cAMP through adenylate cyclase, it is rational to use bronchodilators from both classes when dealing with a refractory case.

Dose

Dogs	:	10 mg/kg, PO, 3 to 4 times daily.
Cats	:	4 mg/kg, PO, 2 to 3 times daily.
Horses	:	10 – 15 mg/kg, PO, 2 times daily.
	:	Up to 15 mg/kg, slow IV. Intravenous solution strength should not exceed 0.015 mg/ml.

Aminophylline

Aminophylline (Theophylline-ethylenediamine) is a theophylline derivative, which is stable in water. It differs from the theophylline only by addition of ethylenediamine to its structure and may have different amounts of molecules of water of hydration. The 100 mg of hydrous aminophylline contains approximately 79 mg of theophylline (anhydrous) and 100 mg of anhydrous aminophylline contains approximately 86 mg of theophylline. On administration, aminophylline dissociates in body and releases the theophylline, which then produces all the pharmacological actions. Due to its water solubility, aminophylline is effective and acts rapidly but it is required in slightly higher doses than theophylline base in order to achieve effective plasma theophylline concentrations.

Dose

Dogs	:	10 mg/kg, PO, IM, or slow IV, 2 to 3 times daily.
Cats	:	5 mg/kg, PO or slow IV, 2 times daily.
Horses	:	5 mg/kg, PO, 2 times daily
	:	9 – 15 mg/kg, diluted in 1L of fluid slow IV over 20-30 min, 2 to 3 times daily.

Etamiphylline

Etamiphylline is the 7-(2-diethylamino ethyl) derivative of theophylline. It is used in both human and veterinary medicine as camsilate salt, which is more water soluble and less irritant to the GI tract than the theophylline. Etamiphylline camsilate has pharmacological properties similar to those of theophylline. It is a smooth muscle relaxant and stimulates both heart and respiration. In contrast to theophylline, it has weak diuretic action.

Etamiphylline camsilate is well absorbed after oral and intramuscular administration in dogs and horses and after intramuscular administration in calves. It is rapidly eliminated from body with elimination half-life of about 100 minutes in horses and 60 minutes in dogs and calves. Its toxicity profile is similar to that of theophylline, but incidence of side effects is much less. Etamiphylline camsilate is used in veterinary practice for treatment of respiratory and cardiac distress.

Dose

Etamiphylline camsilate

Dogs & cats	:	100 – 400 mg (total), PO, 2 to 3 times daily. Dose should be selected on the basis of body weight i.e. < 10 kg = 100 mg, 10-20 kg = 200 mg, 20-30 kg = 300 mg, and >30 kg = 400 mg.
	:	70 – 700 mg (total), SC or IM, 3 times daily. Dose should be selected on the basis of body weight of the animal i.e. <5 kg = 70 mg, 5-10 kg = 140 mg, 10 –20 kg = 280 mg, 20-30 kg = 420 mg, and >30 kg = 700 mg.
Horses	:	Up to 300 mg/100 kg, PO in feed or solution, 3 times daily. Dose may be reduced to lowest effective level after 2 weeks of therapy.
	:	1 – 4 g (total), SC or IM, repeated up to 3 times daily, if required.
Calves	:	700 mg (total), PO, repeated after 3–4 hours if required.
Lambs (≤2.5 kg BW)	:	140 mg (total), PO; dose may be repeated after 3–4 hours, if required.
(>2.5 kg BW)	:	Up to 280 mg (total), PO; dose may be repeated after 3–4 hours if required.

Enprophylline

Enprophylline (Enprofylline, 3-Propylxanthine) is a xanthine derivative. It is more potent than theophylline as a bronchodilator and does not produce obvious effects on CNS, renal function and cerebrovascular resistance. Although it has little effect on gastric secretion, the incidence of GI disturbance is like theophylline and tachycardia even more prominent. It acts primarily as a competitive non-selective phosphodiesterase inhibitor with relatively little activity as a non-selective adenosine receptor antagonist. Enprophylline is primarily used as a bronchodilator in the treatment of asthma and chronic obstructive pulmonary disease. It is also used in the management of cerebrovascular insufficiency, sickle cell disease and diabetic neuropathy. Long-term enprophylline administration may be associated with elevation in liver enzyme levels and unpredictable blood levels.

Other drugs

Several other xanthine drugs are available and can be used as bronchodilators. These include oxytriphylline (Choline

theophyllinate), diprophylline, theophylline sodium glycinate and etophylline (hydroxyethyl theophylline).

IV. Leukotriene Antagonists

Leukotriene antagonists are drugs which act by blocking the formation or activity of spasmogenic leukotrienes. Some drugs are available commercially for the treatment of asthma and some allergic conditions.

Montelukast

Montelukast is a recently introduced leukotriene CysLT1 receptor antagonist. By inhibiting CysLT1 receptor, montelukast blocks the action of leukotrienes D_4, C4 and E4 (see for detail Chapter 29), thereby decreasing bronchoconstriction otherwise caused by the leukotrienes. Montelukast is used clinically in human medicine for the treatment of asthma and to relieve symptoms of seasonal allergies. There is no literature on the clinical use of montelukast in veterinary medicine.

Zafirlukast

Zafirlukast (Accolate) is another leukotriene CysLT1 receptor antagonist with pharmacological actions and therapeutic uses similar to montelukast. It provides beneficial effects in respiratory tract diseases by causing smooth muscle relaxation, decreasing chemotaxis for inflammatory cells and reducing vascular permeability. It effectively prevents antigen-induced and exercise induced bronchospasm and relaxes the airways in mild asthma. Its bronchodilator activity is about 1/3 of salbutamol and its effects are additive with beta$_2$-receptor agonists. In human beings, it is given orally and has good bioavailability. In human medicine, it is often used in conjunction with an inhaled steroid and/or long-acting bronchodilator for the maintenance treatment of asthma in human patients. It may also be used in cats to complement glucocorticoid or bronchodilator therapy in prevention or management of chronic asthma. It should not be used alone in acute asthmatic attacks.

Dose

Cats : 1 – 2 mg/kg, PO, 1 to 2 times daily.

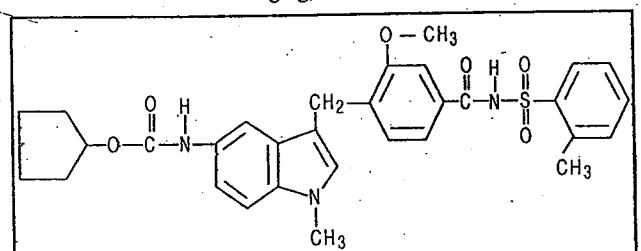


Fig. 46.4. Chemical structure of zafirlukast.

DRUGS USED FOR ALLERGIC AND INFLAMMATORY DISORDERS OF RESPIRATORY TRACT/ASTHMA

Respiratory diseases with a possible allergic aetiology are a poorly defined group of conditions in animals and include feline asthma, pulmonary infiltration with eosinophilia (PIE) in dogs, and chronic obstructive pulmonary disease (COPD) in horses. All are believed to involve an allergic reaction to either inhaled allergens (e.g. mould spores from hay and bedding in horses and house dust mite in cats) or migratory pulmonary parasites. Inflammatory lung diseases have been recorded in swine and other species following inhalation of irritant gases. The symptoms of allergic pulmonary disease can vary from mild intractable coughing to severe respiratory distress and death. Treatment of animal allergic respiratory diseases primarily involves bronchodilators, but the use of anti-allergic (e.g. H_1-receptor antagonists), anti-inflammatory (e.g. NSAIDs and glucocorticoids) and several other agents are also considered useful. Drug therapy of the respiratory tract in animals is most successful when it is based on understanding of the pathophysiology of disease and importance of inflammatory mediators in the pathophysiology of respiratory diseases.

Pathophysiology of Allergic and Inflammatory Diseases of Respiratory Tract / Asthma

Asthma is a pathological state of the lungs characterised by marked bronchospasm, inflammation, airflow obstruction and bronchial hyperactivity often accompanied by increased secretion, mucosal oedema and mucus plugging. The most common allergens implicated in feline asthma syndrome have not been accurately defined but may include human and equine dander (epithelium) and house dust mite. In humans, in addition to allergens (~10%), a variety of trigger factors (e.g. infection, irritants, pollution, exercise, exposure to cold and psychogenic) may be involved. Asthma is now recognised to be a primary inflammatory condition and various mediators released during inflammation are the major contributors in the pathogenesis of this disease.

Asthma has two phases of development, the initial phase and the late phase.

1. Initial phase: The initial phase of asthma occurs abruptly in response to an allergen or non-specific stimulus and is due

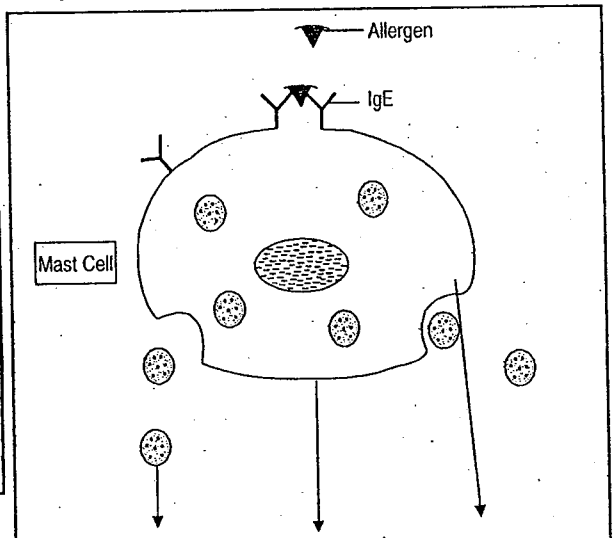


Fig. 46.5. Release of inflammatory mediators by activated mast cells.

mainly to spasm of the bronchial smooth muscle. When allergens come in contact with the allergen-specific IgE, the mast cell is activated. The activated mast cell releases a large number of inflammatory mediators by mechanisms which include release of granule contents (e.g. histamine, proteases and tumour necrosis factor), synthesis of a variety of membrane phospholipid derived factors (e.g. prostaglandins, leukotrienes and platelet activating factor), and production of a number of cytokines (e.g. interleukins) (Figure 46.5). Airway mucosa inflammatory processes may be activated leading to the eosinophilic migration into lung parenchyma and airways and the synthesis of more inflammatory mediators and chemotaxins. The vasodilatation, increased permeability, enhanced adhesiveness to leucocytes and release of various cytokines result in influx of inflammatory cells (e.g. lymphocytes, eosinophils and macrophages) from circulation into tissues, setting the stage for late phase.

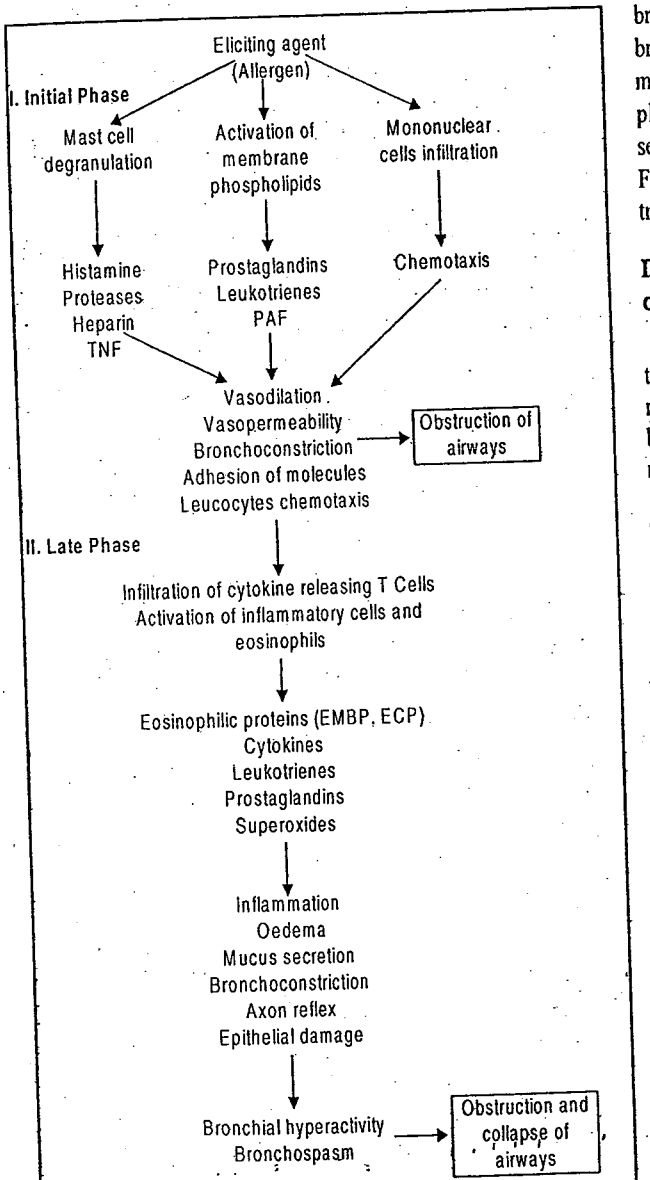


Fig. 46.6. Schematic representation of pathophysiology and sequence of reactions thought to occur in asthma (See text for detail).

Abbreviations: PAF = platelet activating factor; TNF = tumour necrosis factor; ECP = eosinophilic cationic protein; EMBP = eosinophil major basic protein.

In non-allergic asthma, it is possible that irritant may stimulate certain receptors and cause release of neuropeptide mediators from sensory nerve fibre. These neuropeptides then activate mast cells and other cells.

2. Late phase: The late phase occurs at a variable time after exposure to the eliciting stimulus and involves a progressing inflammatory reaction initiated during the initial phase. The infiltration of cytokinin-releasing T cells and eosinophils in asthmatic inflammation in addition to usual inflammatory cells (as seen in other respiratory inflammations) stimulate increase microvascular permeability and mucus secretion and induce chemotaxis. The release of cytotoxic proteins (e.g. eosinophilic proteins) and toxic oxygen radicals further damages the respiratory epithelium and the bronchial tree becomes hypersensitive. Mediators can also inhibit mucociliary function, stimulate afferent nerves endings in mucosa and cause reflex cholinergic bronchoconstriction. Overall, airway obstruction in asthma reflects bronchoconstriction, bronchial wall oedema and accumulation of mucus and cells. As the disease progresses, airways become plugged and ultimately collapse. The pathophysiology and sequence of reactions thought to occur in asthma are given in Figure 46.6. Important mediators and their action on respiratory tract are summarised in Table 46.1.

Drugs Used For Allergic and Inflammatory Disorders of Respiratory Tract/Asthma

The syndrome of chronic bronchial disease/asthma is best treated by breaking the inflammatory cycle and by immediately relieving bronchoconstriction. Thus anti-inflammatory and bronchodilator drugs represent the cornerstone of therapy in many of allergic and inflammatory diseases encountered in animals.

Classification

I. Anti-inflammatory drugs
   1. Corticosteroids
      e.g. dexamethasone, prednisolone and beclomethasone.
   2. Non-steroidal anti-inflammatory drugs
      e.g. flunixin and ketoprofen.

II. Bronchodilators
   1. Adrenergic agonists
      e.g. epinephrine, isoprenaline, clenbuterol and terbutaline.
   2. Muscarinic receptor antagonists
      e.g. atropine, glycopyrronium and ipratropium.
   3. Methylxanthines
      e.g. theophylline and aminophylline.
   4. Leukotriene antagonists
      e.g. montelukast and zafirlukast.

III. Mast cell stabilisers
   e.g. sodium cromoglicate and nedocromil.

IV. Antihistamines
   e.g. chlorphenamine, diphenhydramine, promethazine and cetirizine.

V. Miscellaneous drugs
   1. Serotonin antagonists
      e.g. cyproheptadine and ketotifen.
   2. Decongestants
      e.g. ephedrine, pseudoephedrine and phenylephrine.

Table 46.1: Inflammatory mediators and their important action.

Mediator	Broncho-constriction	Vaso-dilation	Vascular-permeability	Chemo-taxis	Adhesion molecule regulation	Mucus secretion	Cyto-toxicity
Histamine	+	+	+	+		+	
Serotonin*	+		+				
Prostaglandin D_2	+	+	+			+	
Prostaglandin $F_{2\alpha}$	+					+	
Leukotriene B_4				+			
Leukotriene C_4	+	+	+				
Leukotriene D_4	+		+				
Platelet activating factor	+			+			
Tumour necrosis factor (TNF-α)					+		
Proteases	+	+	+				
Interleukin-5 (IL-5)				+			
Interleukin-8 (IL-8)				+			
Macrophage inflammatory protein (MIP-1α)				+			
Eosinophil major basic protein (EMBP)							+
Eosinophilic cationic protein (ECP)							+
Super oxide							+

* = Serotonin does not appear to be an important mediator of human or canine bronchial asthma, but is important in cats.

I. ANTI-INFLAMMATORY DRUGS

1. Corticosteroids

Corticosteroids (Glucocorticoids) remain the most effective long-term therapy for feline bronchial asthma and are the drugs of choice for treatment of canine and feline allergic respiratory diseases. Corticosteroids are very beneficial, especially in cases of severe crippling asthma those are not responsive to the standard bronchodilator therapy. However, these drugs have limited use in status asthmaticus.

Corticosteroids act by stimulating the biosynthesis of lipocortin, a protein that inhibits phospholipase A_2 (see for detail Chapter 51). By preventing formation of prostaglandins, leukotrienes and platelet activating factor, the corticosteroids decrease leucocytes accumulation, prevent and reverse vascular permeability and vasoconstriction, and reduce release of additional mediators. In addition, they induce eosinopenia and lymphopenia, alter macrophages function, modulate immune response, inhibit fibroblast growth and may stabilise mast cell and lysosomal membranes. Thus corticosteroids can modify all phases of inflammation important in bronchial asthma. They also help to restore responsiveness to adrenergic agonists once resistance to them develops and assist to improve mucociliary clearance of airway debris.

The anti-inflammatory potency of glucocorticoids varies with the type of drug and usually requires a lag time before the maximum effect is seen. Although the plasma half-lives of various glucocorticoids range from about 1 hour (prednisolone) to 5 hours (dexamethasone), their duration of action (biological half-life) is much longer. Adverse effects are generally associated with long-term administration of these drugs and may include reduction in resistance to infection and adrenal cortex suppression. Adverse effects in dogs can include dull, dry hair coat, weight gain, vomiting, diarrhoea, elevated liver enzymes, GI ulcerations and behavioural changes. Adverse effects associated with immunosuppressive doses are more common and potentially more severe. The pharmacology and adverse effects of corticosteroids have been discussed in detail in Chapter 51.

In emergencies, intravenous corticosteroids such as dexamethasone, prednisolone or betamethasone may be used, followed by oral prednisolone. Twice daily therapy should be used for the initial control of signs, particularly in patients those are seriously ill, followed by alternate or every third day therapy at the lowest effective dose. Glucocorticoids can also be administered by aerosol for rapid, local effects. Although studies have not proven the efficacy of aerosolisation in small animals, this route may be indicated for treatment of acute exacerbations of disease or for animals intolerant to systemic maintenance glucocorticoid therapy.

Dose

For adjunctive treatment of respiratory disorders

Prednisolone

Dogs & cats : 1 – 2 mg/kg/day, PO, in 2 to 3 divided doses. Every 7-10 days decrease the dose by ¼- ½ as long as signs are controlled. After 3-4 weeks, every other or every third day therapy may be attempted.

Prednisolone sodium succinate

Dogs & cats : 2 – 4 mg/kg, slow IV or IM, 4 to 6 times as adjunctive emergency therapy, followed by oral prednisolone.

Dexamethasone

Dogs & cats : 0.2 – 2 mg/kg, slow IV or IM as adjunctive emergency therapy followed by oral prednisolone.

Triamcinolone

Dogs & cats : 0.25 – 2 mg (total), PO, once daily for 7 days.
: 0.11 – 0.22 mg/kg, PO, IM, or SC, once daily.

Beclomethasone dipropionate inhalant

Dogs & cats : 0.2 mg (total), aerosol inhalation.

2. Non-Steroidal Anti-Inflammatory Drugs

The non-steroidal anti-inflammatory drugs (NSAIDs) are occasionally used in the treatment of respiratory inflammatory diseases, but their exact role in such conditions is still not clear. The NSAIDs effectively block prostaglandins biosynthesis through inhibition of cyclooxygenase enzyme, but they do not appear to have any effect on leukotrienes and other chemical mediators of inflammation (see for detail Chapter 30). They also lack suppression of immune response, which always accompanies the anti-inflammatory actions of corticosteroids. Ventilation, gaseous exchange and pulmonary haemodynamics can all be improved by NSAIDs administration. Flunixin is especially recommended for this purpose. Flunixin meglumine and ketoprofen may be used in the treatment of acute bovine pulmonary emphysema and oedema, and may significantly reduce the mortality due to this condition. The pharmacology of non-steroidal anti-inflammatory drugs has been described in detail in Chapter 30.

II. BRONCHODILATORS

Bronchodilators are often used with or without concurrent corticosteroid therapy for the control of chronic bronchitis in dogs and asthma syndrome in cats. Short acting beta$_2$-adrenoceptor agonists, such as clenbuterol are the first line treatment for the acute asthma symptoms. Non-selective beta adrenoceptor blockers such as epinephrine and isoprenaline have efficacy similar to that of selective beta$_2$-adrenergic receptor agonists, but are less used nowadays due to concerns regarding excessive cardiac stimulation. Muscarinic receptor antagonists such as ipratropium bromide are often used in adjunctive therapy with beta$_2$-adrenoceptor agonists to provide additional benefit in patients with moderate or severe symptoms. Antimuscarinic bronchodilators can also be used alone as the first line treatment if the selective beta$_2$-adrenoceptor agonists cannot be used or are contraindicated in some individuals. Leukotriene antagonists such as zafirlukast are an alternative to the primary drugs, but are not preferred. The pharmacology of bronchodilator drugs have been discussed earlier in the present chapter.

III. MAST CELL STABILISERS

Sodium cromoglicate

Cromoglicic acid (Cromolyn, cromoglicate, cromoglycate) is a synthetic chromone derivative, which inhibits degranulation of mast cells by trigger stimuli. Cromoglicic acid is commonly marketed as sodium salt and is known by name sodium cromoglicate or cromolyn sodium. Although devoid of the bronchodilatory property, sodium cromoglicate inhibits antigen-induced bronchospasm as well as release of histamine and other mediators from the sensitised mast cells.

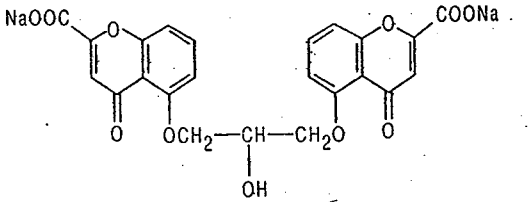


Fig. 46.7. Chemical structure of sodium cromoglicate.

Mechanism of action

Sodium cromoglicate inhibits mast cell degranulation by stabilising the mast cell membrane. The exact mechanism by which sodium cromoglicate and other mast cell stabilizers act is not clearly known. It is postulated that they block a calcium channel essential for mast cell degranulation, stabilizing the cell and thereby preventing the release of histamine and related mediators. One suspected pharmacodynamic mechanism is the blocking of IgE-regulated calcium channels. Without intracellular calcium, the histamine vesicles cannot fuse to the cell membrane and degranulate. There is evidence that it depresses also exaggerated neuronal reflexes which are triggered by stimulation of the irritant receptors and may inhibit release of preformed T-cells cytokines. It also inhibits chemotaxis of inflammatory cells.

Pharmacological effects

Long-term treatment of sodium cromoglicate decreases cellular inflammatory response, bronchial hyperactivity and bronchospasm induced by allergens, irritants, cold air and exercise. However, it does not interfere with antigen-antibody reaction and also does not antagonise bronchoconstrictor action of histamine, acetylcholine and leukotrienes. Therefore, it is not effective if given during an asthmatic attack or existing bronchoconstriction.

Pharmacokinetics

Sodium cromoglicate is poorly absorbed from the GI tract, so it is administered by aerosol. After administration, only a small fraction of the inhaled drug is absorbed systemically, which is rapidly excreted unchanged in urine and bile. The drug has a short half-life (man = 1.5 hours). Thus, effective therapy is dependent on frequent aerosolisation, which limits its utility in the treatment of animals' diseases.

Side effects/Adverse effects

Sodium cromoglicate has no serious side/adverse effects. In human beings, bronchospasm, throat irritation, cough and rashes have been reported in some patients.

Clinical uses

Cromoglicate is most useful as a preventive drug prior to activation of inflammatory cells. It is used primarily as a prophylactic therapy of allergic disease. In human medicine, cromoglicate is used in the management of asthma. In veterinary practice, it is used for prophylaxis of chronic obstructive pulmonary disease (COPD) in horses, where it is administered by inhalation using a facemask prior to expected exposure to an allergen. The length of protection is dependent on the number of consecutive days that the drug is administered. Four days of sodium cromoglicate administration may protect the horse for up to 20 days. It is commercially available in different formulations such as nasal spray for rhinitis, nebulizer solution or inhalant for asthma, ophthalmic drops for conjunctivitis, and oral concentrate for dermatographic urticaria, ulcerative colitis and food allergy.

Dose

Horses : 80 mg (total), by inhalation (using special nebuliser and face mask), once daily for 1 to 4 days. The 10-15 minutes period nebulisation is generally well accepted by horses.

Nedocromil sodium

Nedocromil sodium is a chromone derivative having biological and chemical properties similar to those of cromoglicate. It is more potent and probably more effective than cromoglicate. In human patients, it is requires twice daily dosing (nebulisation). Its use in veterinary medicine requires investigations.

Dose

Horses : 80 mg (total), by inhalation (using special nebuliser and face mask), 2 times daily for 1 week, then once daily.

IV. ANTIHISTAMINES

Histamine H_1-receptor antagonists (antihistamines) diminish or abolish the main actions of histamine in body by competitive blockade of histamine receptors. The role of histamine in hypersensitivity reactions and as an inflammagen is well known. However, histamine is only one of many autacoids involved in hypersensitivity reactions or asthma; so antihistamines have limited use in the treatment of allergic respiratory diseases in animals. In animals with chronic disease, the use of H_1-receptor blockers may be harmful because of their inhibitory effects on airway secretions. Several of these agents, however, do exert some central effects on the cough centre and may reduce bronchospasm. For these reasons, antihistamines like diphenhydramine and promethazine are often incorporated in compound cough remedies. Some reports have suggested that newer, non-sedating antihistamines such as cetirizine and terfenadine are reasonably effective in mild atopic asthma in human beings. Their use in animals, however, requires further studies.

Dose

Promethazine HCl

Dogs : 0.2 – 1 mg/kg, PO or SC, 2 to 3 times daily.

Diphenhydramine

Dogs : 2 mg/kg, PO, 3 times daily.

V. MISCELLANEOUS DRUGS

1. Serotonin Antagonists

The clinical efficacy of serotonin receptor antagonists in the management of small animal bronchial disease has not been studied extensively despite the apparent sensitivity of feline bronchial smooth muscle to serotonin.

Cyproheptadine

Cyproheptadine is an antihistamine with serotonin antagonist and calcium-channel blocking properties. It may be used for prophylaxis in feline asthma. However, its use is recommended only when other control methods have failed. The most likely adverse effects seen with cyproheptadine are related to its CNS depressant (sedation) and anticholinergic (xerostomia) actions. The pharmacology of cyproheptadine has been discussed in Chapter 27.

Dose

For prophylaxis in feline asthma

Cats : 0.3 – 0.5 mg/kg, PO, 3 times daily.

Ketotifen

Ketotifen is a serotonin antagonist with some degree of H_1-receptor antagonism and cromoglicate like mast cell stabilizing action. Phosphodiesterase inhibition, restriction of Ca^{++} entry, and leukotrienes C_4 and D_4 (LTC_4/LTD_4) antagonism have also been demonstrated with ketotifen. Ketotifen is not a bronchodilator, but it produces some reduction in delayed phase of airway obstruction in human asthma. It is given orally and may require 3-4 weeks to produce desirable effect. Drowsiness and sedation are its primary side effects. In veterinary medicine, detailed information on ketotifen is not available.

2. Decongestants

Sympathomimetics with alpha$_1$-adrenoceptor agonistic activity produce vasoconstriction in mucous membranes, which reduces swelling and oedema. These drugs are used topically as nasal decongestants in allergic and viral rhinitis and systemically as respiratory tract decongestants. Intermittent topical therapy is preferred to facilitate breathing and to reduce the volume of nasal discharge. Examples of alpha$_1$-adrenoceptor agonists used for this purpose include ephedrine, pseudoephedrine, phenylephrine, phenylpropanolamine and naphazoline.

The decongestant activity of ephedrine, a mixed acting non-catecholamine (Chapter 11), is partially dependent on norepinephrine release from adrenergic nerve terminals. Therefore, its prolonged use may deplete norepinephrine storage granules and the animal becomes refractory to its effects. In addition, rebound condition may result from secondary beta-adrenoceptor effects as receptors upregulate. Overall, the use of decongestants in veterinary medicine is not common. The pharmacology, side/adverse effects and doses of some commonly used decongestants have been given in Chapter 11.

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CHAPTER 47 ANALEPTICS AND OXYGEN THERAPY

Analeptics
- Classification
- Specific respiratory stimulants
- Non-specific respiratory stimulants
  - Methylxanthines
  - Opioid antagonists
  - Gases and respiratory irritants
  - Alpha-adrenoceptor antagonists

Oxygen therapy
- Hypoxia
- Oxygen supplementation

ANALEPTICS

Analeptics (Greek: *analeptikos* = restorative) are drugs which stimulate respiration and can have resuscitation value in respiratory depression, coma or unconsciousness. Analeptic acts mainly on brain stem and stimulate the vital centres located in medulla; therefore, they are sometimes also called 'brain stem stimulants' (see Chapter 25). In addition to restoring respiratory function, analeptics may restore depressed vasomotor and cerebral functions including consciousness. However, analeptics are not routinely used in therapeutics due to a number of shortcomings associated with their use. In brief, their advantages and limitations are given below:

Advantages

a. They are useful as expedient measure in hypnotic and sedative drug poisoning until mechanical ventilation is instituted.
b. They are useful in acute respiratory insufficiency like suffocation due to drowning.
c. They are useful in apnoea in pre-term and newborn animals.
d. They are useful to reverse respiratory depression associated with general anaesthesia (the clinical usefulness of analeptics in lessening deep anaesthesia is debatable).
e. They are useful in respiratory failure due to removal of hypoxic drive as a result of giving too high oxygen concentration to patients with chronic lung disease.

Limitations

a. All respiratory analeptics have narrow margin of safety and may produce clonic convulsion in doses slightly higher than those required for respiratory stimulation.
b. Their action is not confined to the respiratory centre alone, but other centres like vagal, vomiting and vasomotor centres may also be stimulated.
c. Post-tictal depression often adds to the existing depression, which may worsen the condition.
d. While analeptics temporarily increase tidal and minute volumes, they also increase basal metabolism and oxygen utilisation that negate the beneficial effect.
e. The clinical effectiveness of analeptics in lessening deep anaesthesia and management of poisoning by CNS depressant is dubious.
f. They are not dependable for long-time support and may jeopardise ultimate outcome and survival.
g. They are not active in deep coma, except in near convulsive doses.

Respiratory analeptics should not be used as an alternative to patient management. In drug induced respiratory depression, maintenance of an adequate airway and airflow by intubation and positive pressure ventilation are much more successful and safe. If the analeptic therapy is considered essential in a life-threatening emergency (e.g. respiratory arrest), then it should be administered by slow IV infusion to the effect (until breathing restarts). Then mechanical support to respiration and other measures to improve circulation should be instituted and infusion of analeptic stopped.

Classification

I. Specific respiratory stimulants/Analeptics
   e.g. doxapram, nikethamide, bemegride, cropropamide, cotethamide, amiphenazole and etamivan.
II. Non-specific respiratory stimulants/Miscellaneous analeptics
   1. Methylxanthines
      e.g. diprophylline, etamiphylline and aminophylline.
   2. Opioid antagonists
      e.g. naloxone, butorphanol and buprenorphine.
   3. Gases and respiratory irritants
      e.g. carbon dioxide and ammonia.
   4. Alpha-adrenoceptor antagonists
      e.g. yohimbine.

I. SPECIFIC RESPIRATORY STIMULANTS/ANALEPTICS

Doxapram

Doxapram HCl is a white to off-white, odourless, crystalline powder that is soluble in water, sparingly soluble in alcohol and practically insoluble in ether. Doxapram is one of very few analeptics still used in veterinary medicine to any significant extent.

Pharmacological effects

Doxapram is a general stimulant, but at low doses it is more selective for the respiratory centre than are other analeptics. The effects of respiratory stimulation are generally a result of direct excitation of neurons of the respiratory centre and indirectly through reflex activation of the carotid and aortic chemoreceptors, which in turn stimulate respiratory centre in the brain stem. Transient increase in respiratory rate and volume usually occurs, but increases in artificial oxygenation do not ensue. This is because doxapram generally increases the work associated with respiration with resultant increased oxygen consumption and carbon dioxide production. Its action is transient and animal quickly relapses into respiratory depression, if the respiration restored by doxapram is not maintained by artificial ventilation.

Doxapram is considered superior to other analeptics. The increase in respiratory minute volume after IV administration of doxapram occurs to the extent of 200% within 1 minute in dogs. The ventilatory and cardiovascular stimulatory effects generally occur within one circulation time of the drug. Stimulation of other portions of CNS occurs only when high dose levels are used. Convulsion or altered EEG patterns are rarely seen with therapeutic doses. Doxapram increases catecholamine concentration through activation of sympathetic nervous system by peripheral chemoreceptors.

Pharmacokinetics

After IV administration, doxapram produces analeptic action within 1-2 minutes that lasts for 5 to 6 minutes. In dogs, doxapram is well distributed into tissues, is rapidly metabolised and excreted mainly as metabolites in urine within 24-48 hours. Small quantities of metabolites may be excreted up to 120 hours after dosing.

Side effects/Adverse effects

Side effects indicative of subconvulsive CNS stimulation are common with doxapram. Hypertension, arrhythmias, coughing and hyperventilation leading to respiratory alkalosis has been reported. Vomiting, itching, tremors, muscle rigidity, sweating and hyperpyrexia may also occur. These effects are more probable with repeated or high doses. Symptoms of overdosage include hypertension, tachycardia and generalised CNS excitation including seizures.

Treatment: Treatment of doxapram toxicity is only supportive. Drugs such as short acting IV barbiturates may be used to help decrease CNS hyperactivity. Oxygen therapy may be instituted.

Contraindications and precautions

Doxapram should be used with extreme caution in patients with cerebral oedema, increased CSF pressure, phaeochromocytoma and those sedated with morphine. It should not be used in patients suffering from epilepsy and hypertension.

Drug interactions

As doxapram increases concentration of catecholamines, its use should be delayed for about 10 minutes after discontinuation of anaesthetics, especially the agents which sensitise myocardium to catecholamines (e.g. halothane and enflurane). Doxapram used with sympathomimetics may significantly enhance blood pressure. Doxapram may mask effects of muscle relaxant drugs.

Clinical uses

Doxapram may be used clinically to stimulate respiration during and after general anaesthesia in dogs, cats and horses. It is also used to promote respiration in apnoeic newborn and premature young ones and to remove respiratory depression associated with sedative and hypnotic drugs. In humans it may be useful for treating respiratory depression in patients who have taken excessive doses of drugs such as buprenorphine and have failed to respond adequately to treatment with naloxone.

Dose

Inhalation anaesthesia

Dogs & cats : 1 – 2 mg/kg, IV.

Intravenous anaesthesia

Dogs & cats : 2 – 5 mg/kg, IV.

Cattle and swine : 5 – 10 mg/kg, IV.

Horses : 0.5 – 1 mg/kg, IV at 5 minutes interval.

For apnoea in newborns (to stimulate respirations)

Puppies : 1 – 5 mg (total), IV, SC, or by sublingual application (1-2 drops under tongue).

Kittens : 1 – 2 mg (total), IV, SC, or sublingual application.

Lambs : 5 – 10 mg (total), IV, SC, or by sublingual application.

Calves and foals : 40 – 100 mg (total), IV, SC, or by sublingual application.

Nikethamide

Nikethamide, widely known by its former trade name of coramine, is a synthetic pyridine derivative having weak analeptic action. It stimulates chemoreceptor activity in the carotid and aortic bodies, which reflexly stimulate respiratory centre primarily and vasoconstrictor and vagal centres secondarily. An excessive dose of nikethamide stimulates the cerebral cortex and spinal cord resulting in convulsion. Respiratory stimulation by nikethamide is short lasting and is followed by a period of depression. It is similar to doxapram in action, but is less selective that reduces its value as an effective analeptic agent.

Nikethamide is absorbed readily following any route of administration. It is injected ordinarily by IM route but is most potent when given intravenously. After metabolism, it is converted to nicotinamide and excreted as N-methylnicotinamide. Nikethamide has low toxicity, but is also less efficacious and less selective than doxapram. It is rarely used nowadays.

Dose

Dogs : 22 – 44 mg/kg, PO, IV, IM, or SC.

Bemegride

Bemegride (Megimide) is a general CNS stimulant having actions similar to pentylenetetrazol, but with a wider margin of safety. Its analeptic effect is rapid in onset and of short duration. It is chemically related to barbiturates, but is not a specific barbiturate antagonist. Large doses of bemegride produce muscular twitching, tremors and convulsions. Convulsive seizures occur more readily in cats than in dogs. It is occasionally used in veterinary medicine to stimulate acutely depressed respiration in barbiturate toxicity.

Dose

Dogs & cats : 15 – 20 mg/kg, IV.

Cropropamide and Crotethamide

A mixture of equal parts of cropropamide and crotethamide (crotetamide), known as procethamide, is used to stimulate respiration in newborn animals or during deep anaesthesia. Chemically, these compounds are similar to nikethamide and therefore probably act as stimulant to carotid and aortic body chemoreceptors. However, these compounds have more tendencies to produce convulsions than doxapram and hence are less used clinically.

Dose

For neonate apnoea

All species : Few drops of syrup containing active ingredients are placed on the tongue of neonate. It may be repeated at 30 seconds for 2 to 3 times until response is obtained. Care should be taken not to administer more than 3 doses.

Amiphenazole

Amiphenazole is an analeptic traditionally considered an antidote to barbiturate or opiate poisoning, when used in combination with bemegride. It was also considered useful in the treatment of respiratory depression caused by other CNS depressants. It is still occasionally used in some countries, but has largely been replaced by more effective respiratory stimulants such as doxapram and specific opioid antagonists such as naloxone.

Etamivan

Etamivan (Ethamivan) is another analeptic related to nikethamide. It is considered useful in the treatment of hypoventilatory states like barbiturate toxicity. It may also be used as a temporary measure to correct acute respiratory insufficiency in patients with chronic obstructive pulmonary disease. It has now largely fallen into disuse.

Other drugs

Some other less commonly used analeptics include almitrine, dimefline and mepixanox (pimexone). Zacopride, an anti-emetic, anxiolytic and nootropic agent, has shown pro-respiratory effects, both by reducing sleep apnoea and by reversing opioid-induced respiratory depression in animal studies.

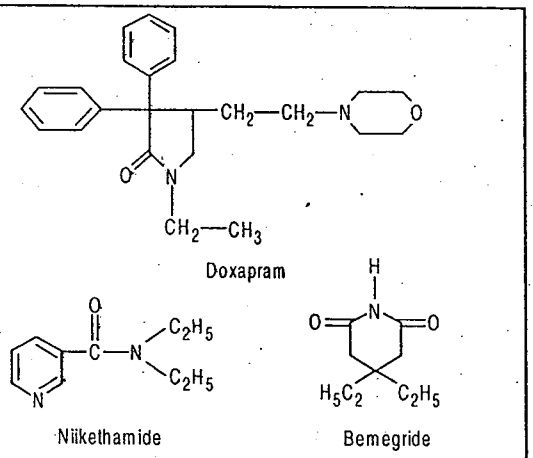


Fig. 47.1. Chemical structures of selected analeptics.

II. NON-SPECIFIC RESPIRATORY STIMULANTS / MISCELLANEOUS ANALEPTICS

1. Methylxanthines

The methylxanthines such as diprophylline, etamiphylline and aminophylline are non-specific CNS stimulants. They increase respiratory drive by altering the respiratory centre's sensitivity to carbon dioxide and also cause bronchodilation. Methylxanthines can be used as respiratory stimulants in neonates or in respiratory diseases where bronchodilation may be beneficial. The detailed pharmacology of methylxanthines has been discussed in Chapter 46.

Dose

Etamiphylline camsylate

Calves : 700 mg (total), PO; dose may be repeated after 3 to 4 hours, if required.

Lambs (<2.5 kg) : 140 mg (total), PO. Dose may be repeated after 3 to 4 hours, if required.

(>2.5 kg) : Up to 280 mg (total), PO. Dose may be repeated after 3 to 4 hours, if required.

Piglets : 70 – 140 mg, PO.

2. Opioid Antagonists

Opioid antagonists should be used to reverse respiratory depression induced by opiates. Naloxone is a pure antagonist and, therefore, the most effective reversal agent for opiate-induced respiratory depression. Some other drugs like butorphanol and buprenorphine (opioid agonist/antagonists) can also reverse the sedative and respiratory depression induced by opiates. The detailed pharmacology of opioid antagonists has been discussed in Chapter 24.

3. Gases and Respiratory Irritants

Carbon dioxide

Carbon dioxide is considered to be one of the most potent stimulants of respiration. Inhalation of carbon dioxide influences carotid and aortic bodies, which reflexly stimulate respiratory centre. In addition, carbon dioxide has some direct effect on the respiratory centre. The clinical use of carbon dioxide gas to stimulate depressed respiration is not a common practice in animals.

Respiratory irritants

Local respiratory irritants like ammonia gas cause reflex stimulation of the respiratory centre. Inhalation of ammonia gas evolved from stopper of a bottle of strong solution of ammonia or from ammonium carbonate may improve both respiratory and circulatory movement, provided CNS depression is not deep.

4. Alpha-Adrenoceptor Antagonists

Selective alpha$_2$-adrenoceptor antagonists like yohimbine and non-selective alpha-adrenoceptor antagonists like tolazoline can be used to reverse sedative and respiratory depression effects induced by alpha$_2$-adrenoceptor agonists.

OXYGEN THERAPY

Oxygen is an essential component of all types of life with no warm-blooded animal surviving for longer than few minutes in absence of oxygen. Hypoxia or insufficient oxygenation of tissue, regardless of its cause, is a life-threatening condition and generally requires immediate oxygen therapy because damages from hypoxia are rapid and often irreversible.

Hypoxia

Hypoxia may be defined as decreased concentration of oxygen in the inspired air, blood, or tissues short of anoxia. Hypoxia may be pre-pulmonary, pulmonary or post-pulmonary. Pre-pulmonary hypoxia results from inadequate delivery of oxygen to the lungs and is usually seen in high altitudes (low oxygen pressure/concentration), airway obstruction (e.g. laryngospasm and bronchospasm), muscular weakness (disease or neuromuscular blockade), or impaired delivery drive (e.g. CNS disorder, opioids and general anaesthetics). Pulmonary hypoxia may occur due to abnormal pulmonary function despite normal delivery of oxygen

to the lungs (e.g. asthma, emphysema, fibrosis and oedema). Despite normal PaO_2, inadequate delivery or utilisation of oxygen by tissues results in post-pulmonary hypoxia (e.g. anaemia, carbon monoxide and cyanide poisoning and hyperpyrexia). Many of these causes of hypoxia respond to oxygen therapy.

OXYGEN SUPPLEMENTATION

Oxygen

Oxygen is an ideal therapeutic agent as it is easy to administer, readily available, and if used correctly, relatively safe. Oxygen is given as a temporary therapy to prevent tissue hypoxia and is not a substitute for definitive treatment of the basic cause underlying.

Pharmacological effects

Inhalation of adequate concentration of oxygen in inspired air reverses effects of hypoxia and prevents complications associated with development of hypoxia. When animals are responsive to oxygen, small changes in oxygen supplementation may produce dramatic changes. However, inhalation of pure oxygen (100%) at 1 atmospheric or above causes a small degree of respiratory depression in normal subjects as a result of loss of tonic chemoreceptor activity. It is, therefore, advisable to administer a mixture of 95% oxygen and 5% carbon dioxide. The addition of carbon dioxide promotes hyperaemia of respiratory tract and increases volume and fluidity of secretions, and more importantly provides a physiological stimulation of the respiratory centre.

Side effects/Adverse effect

Adverse effects of oxygen therapy at recommended concentrations are relatively uncommon. However, administration of 100% oxygen for longer than 12 hours or of 80% to 90% oxygen for longer than 18 hours can contribute to alterations in pulmonary function and signs of oxygen toxicity like increased secretions, pulmonary oedema and generalised atelectasis. Oxygen toxicity is related, in part, to the production of reactive species such as superoxide anion, singlet oxygen, hydrogen radical and hydrogen peroxide. The oxidative damage initiated by these substances results in lipid peroxidation and membrane damage. Cell injury and death are presumed to result from loss of membrane integrity. Oxygen toxicity is often difficult to recognise because the toxic signs can be similar to those seen in hypoxia.

Treatment: There is no specific treatment to oxygen toxicity. It is reversible in the early stages, if oxygen is discontinued or its concentration is decreased. In advanced stages, decreasing of dangerously high levels of inspired gas with supportive measure may help in the recovery.

Clinical uses

Oxygen therapy is indicated in a wide variety of clinical conditions whenever alveolar ventilation is critically compromised. High flow oxygen is indicated for use in resuscitation, major trauma, anaphylaxis, shock, active convulsions and hypothermia. Other important uses of oxygen therapy may include carbon monoxide poisoning and gas gangrene. In carbon monoxide poisoning, increased oxygen concentration in lungs helps to displace carbon monoxide from the heme group of haemoglobin. In gas gangrene, increasing the partial pressure of oxygen kills the anaerobic bacteria which cause gas gangrene.

Most animals requiring oxygen supplementation should preferably be hospitalised and monitored closely for all vital parameters including body temperature, pulse rate and respiratory rate. Oxygen should be delivered by means of a mask, endotracheal tube, nasal catheter, respirator, or an oxygen cage. Using the masks is perhaps the easiest and least invasive techniques for oxygen supplementation in animals. Discontinuation of oxygen therapy is not done abruptly, but should be weaned off (tapered off) slowly over a period of 24-48 hours depending on the response and the condition of animal.

Dose

A concentration of 40-60% oxygen in inspired air is regarded as optimal for most species. Flow rates of 2-5 L/minute for small animals and about 12 L/minute for large animals are used. In emergency situations, 100% oxygen for brief periods may be used to save the life of animal.

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SECTION-IX

DRUGS ACTING ON ENDOCRINE SYSTEM

कृपया बेचा खरीदा
पुस्तक जमा कर गये.

CHAPTER 48 GENERAL CONSIDERATIONS

Hormones
Divisions of endocrine system
Chemistry of hormones
Synthesis, storage and secretion of hormones
Mechanisms and control of hormonal action

The term 'endocrine' refers to the system of ductless glands which synthesize and secrete hormones directly into the bloodstream to regulate and coordinate various body functions. In addition to the specialized endocrine organs, many other organs and tissues such as kidneys, liver and gonads have secondary endocrine functions as they also synthesize and secret certain hormones. The endocrine system is coordinated by the nervous system which synthesizes certain factors and/or sends information for the release of hormones from endocrine tissues. Therefore, endocrine system is more precisely a 'neuroendocrine system'. The coordinated activity of these two major systems viz. nervous and endocrine is essentially required for the stability of internal environment (homeostasis) in the body.

Hormones

A hormone (Greek word *hormaein* = to stir up) is a chemical substance that is produced by the endocrine (ductless) glands or specific cells in body and is transported through circulation to act on its target cells. Hormones carry messages like neurotransmitters, but unlike neurotransmitters they act slowly and convey messages to organs or tissues those may be located distantly in the body. Hormones are involved mainly in the control of physiological functions including metabolism, growth, development and reproduction. They may have relatively few functions at specific sites (e.g. oxytocin) or affect all or almost all cells of the body (e.g. growth hormone). Hormones may be directly responsible for changes in an organ (e.g. epinephrine) or act indirectly to cause production of other hormones (e.g. anterior pituitary hormones).

Drug therapy for the endocrine system is implemented mainly either to replace a hormone deficiency when the gland function is inadequate or to reduce or prevent effects of an overactive hormone. Drugs are used also as diagnostic tools for evaluation of several endocrine disorders. Understanding the proper uses of drugs depends on the thorough knowledge of normal physiology of each endocrine system, including mechanism of control and behaviour of target tissues.

Divisions of Endocrine System

The endocrine system consists of many important endocrine glands. Major endocrine glands and important hormones released by them have been categorised as below:

I. Pituitary
  1. Anterior pituitary
    a. Growth hormone
    b. Prolactin
    c. Thyroid stimulating hormone/Thyrotropin
    d. Follicle stimulating hormone
    e. Luteinising hormone
    f. Adrenocorticotropin hormone/Corticotropin
  2. Posterior pituitary
    a. Antidiuretic hormone/Vasopressin
    b. Oxytocin
II. Thyroid
  a. Thyroxine (T_4)
  b. Triiodothyronine (T_3)
  c. Calcitonin
III. Parathyroid gland
  a. Parathormone
IV. Adrenal gland
  1. Adrenal cortex
    a. Glucocorticoids
    b. Mineralocorticoids
    c. Sex steroids
  2. Adrenal medulla
    a. Epinephrine
    b. Norepinephrine
V. Gonads
  1. Ovaries
    a. Oestrogens
    b. Progesterone
  2. Testes
    a. Androgens
VI. Pancreas/Islets of Langerhans in Pancreas
  a. Insulin
  b. Glucagon

In addition to above-mentioned hormones, hypothalamus, which is a part of the CNS and not a gland, produces many releasing and inhibitory hormones which control secretions of anterior pituitary hormones (See Chapter 49). Placenta also synthesises and secretes some hormones like equine chorionic

gonadotropin, oestrogens and progesterons, which promote growth of the maternal sex organs and some foetal tissues. Similarly, stomach releases gastrin, ghrelin, neuropeptide Y, somatostatin and endothelin, duodenum releases secretin and cholecystokinin, liver releases insulin-like growth factor and thrombopoietin, kidney releases erythropoietin, pregnant uterus releases relaxin, and heart releases atrial-natriuretic peptide.

Chemistry of Hormones

Nature uses a diverse spectrum of molecules as hormones. The hormones existing in domestic animals belong to three main chemical classes – proteins and peptides, amino acid derivatives and steroids. Members of these chemical classes have many properties in common.

a. Proteins and peptides: Protein and peptide hormones are products of translation carried by the m-RNA. They vary considerably in size and post-translational modifications, ranging from peptides as short as three amino acids to large multi-subunit glycoproteins. The posterior pituitary hormones are peptides, the anterior pituitary hormones are either proteins or large polypeptides, and the insulin, glucagon and parathormone are all large polypeptides. The half-life of circulating peptide hormones is only a few minutes.

b. Derivatives of amino acids: Some amino acids through metabolic conversion provide important hormones. These include epinephrine and norepinephrine, the main catecholamine hormones of adrenal medulla, derived from amino acid phenylalanine or tyrosine. The two metabolic thyroid hormones, thyroxine and triiodothyronine, are iodinated form of tyrosine derivatives. Tryptophan is the precursor to the pineal hormone melatonin. The circulating half-life of amino acid derived hormone may vary from a few minutes (e.g. catecholamines) to few hours or days (e.g. thyroid hormones).

c. Steroid hormones: Steroidal hormones are lipids and, more specifically, derivatives of cholesterol. Hormones secreted by adrenal cortex, gonads and placenta have a common four-ring 17-carbon skeleton based on the steroid nucleus, similar to that of cholesterol. Steroid hormones are typically eliminated by inactivating metabolic transformations and excretion in urine or bile. The rates of metabolism of steroid hormones in the circulation vary but generally have their half life in hours.

Synthesis, Storage and Secretion of Hormones

There is no single way in which all the endocrine glands produce, store and secrete their hormones. However, several general patterns occur for most of the hormones.

a. Peptide and protein hormones: Most protein and peptide hormones require the transcription of specific gene(s) located on DNA. For this reason, first the gene(s) regulating the protein and polypeptide hormones synthesis forms the mRNA in the cell nucleus. The newly synthesised m-RNA then migrates from nucleus to the cytoplasm, where ribosomes are located. The ribosomes in stepwise manner translate the information carried by the m-RNA for the synthesis of new protein. The peptide and protein hormones are generally synthesized first as an initial large protein called a preprohormone, which is further cleaved to form a smaller protein called the prohormone; the cleavage of proteins occurs in the endoplasmic reticulum. The prohormone is then transported in the endoplasmic reticulum transport vesicles to the Golgi apparatus, where it is further cleaved to form the final active protein hormone. Once synthesized, the hormone molecules are stored in secretory vesicles or secretory granules in the cytoplasmic compartment of the endocrine cell (Figure 48.1). Although some of the hormones are secreted on a continuous basis (called constitutive secretion), most are secreted through the process of exocytosis in response to increased intracellular calcium generated by a specific signal such as a nerve impulse or another hormone (called regulated secretion). The synthesis, storage and release of various proteins/peptides have been explained in detail in Chapter 9.

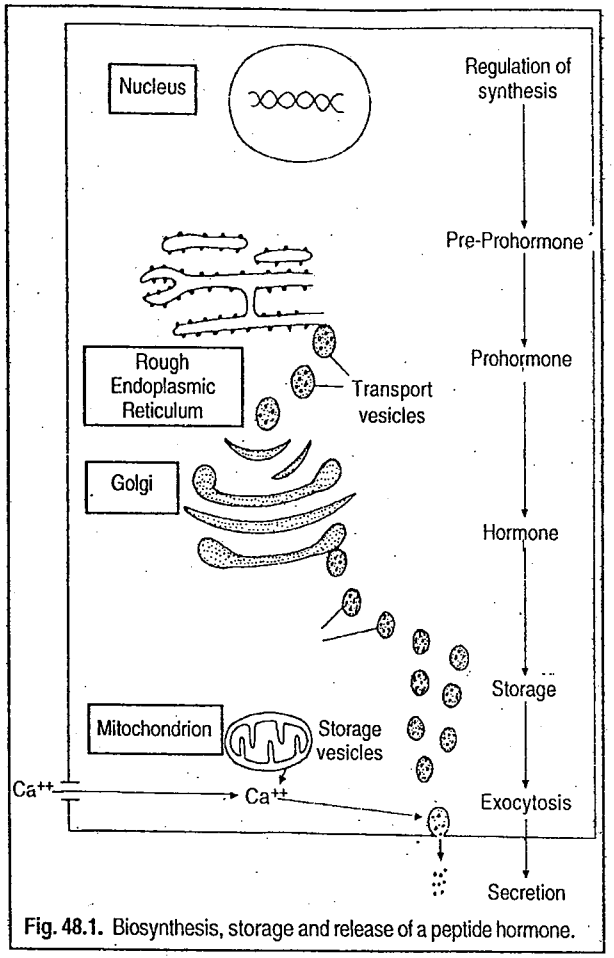


Fig. 48.1. Biosynthesis, storage and release of a peptide hormone.

b. Derivatives of amino acid tyrosine: The thyroid and adrenal medullary hormones are biosynthesised in cytoplasmic compartments of the glandular cells in a stepwise manner by actions of enzymes. After synthesis, the adrenal medullary hormones (e.g. epinephrine and norepinephrine) are packed into the pre-formed vesicles and stored until they are to be secreted by exocytosis (see for detail Chapter 10). The thyroid hormones (T_3 and T_4), in contrast, are initially formed as component parts of a large protein molecule called thyroglobulin, which is then stored in large follicles within the thyroid gland. When required, specific enzyme system within thyroid

Fig.

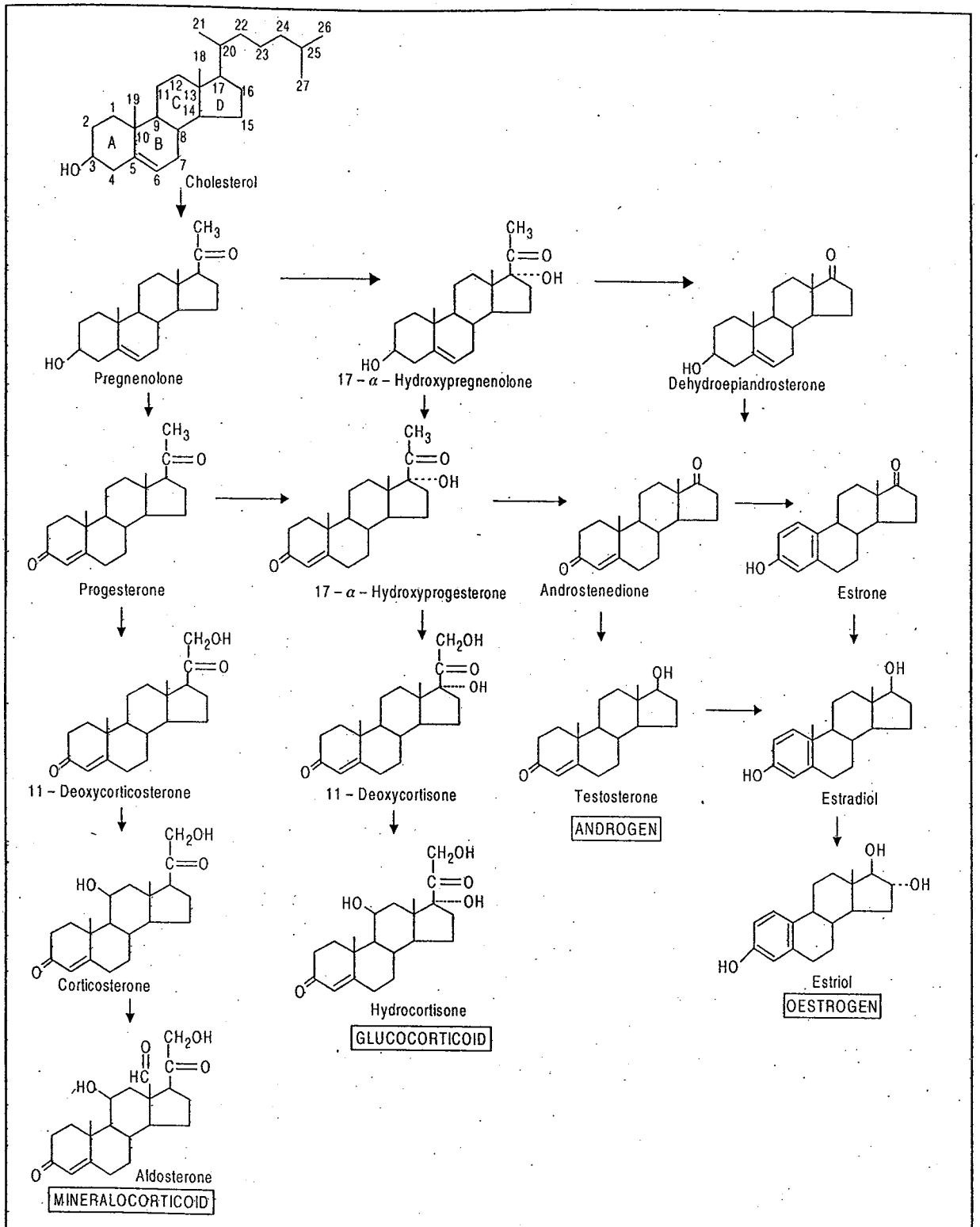


Fig. 48.2. Schematic representation of various pathways involved in the production of major steroid hormones.

glandular cells cleaves the thyroglobulin molecule to form thyroid hormones, which are then secreted into the blood. The synthesis and release of thyroid hormones have been described in Chapter 50. The action of catecholamines is terminated either by process of re-uptake or by enzymatic metabolism. Thyroid hormones are inactivated primarily by intracellular deiodinases.

c. ***Steroid hormones:*** Steroid hormones are formed mainly from cholesterol, which is synthesised by liver. Normally the amount of steroid hormones present in glandular cells is quite small, but large amounts of precursor molecules especially cholesterol and various intermediates between cholesterol and final hormone are present in these cells. On appropriate stimulation, enzymes in these cells can within minutes cause necessary chemical conversions to the final hormones (Figure 48.2). The type of steroid hormone synthesised is dependent on the presence of specific enzymes within the particular cell.

These hormones are secreted immediately after formation by simple diffusion because of their lipophilic nature. There is no provision for the storage of steroid hormones within cells; therefore, the rate of hormone secretion is controlled mainly by the rate of synthesis. The synthesis and release of steroid hormones have been discussed also in Chapter 51.

Mechanisms and Control of Hormonal Action

The endocrine hormones after their release are transported by blood vascular system to affect other tissues. The protein hormones are hydrophilic and are carried in plasma in dissolved form, whereas steroid and thyroid hormones are lipophilic and carried in plasma in association with various types of plasma proteins (because of their limited solubility in aqueous solutions).

The hormones most often do not act directly on the intracellular machinery, but require interaction with specific hormone receptors. Protein hormones have specific receptors on target tissues plasma membrane, whereas steroids have specific receptors within the cytoplasm or nucleus. The proteins or peptide hormones require an intermediary second messenger to act on their behalf, because they are unable to penetrate the plasma membrane at the surface of the cell. The interaction of hormone and receptor typically triggers a cascade of secondary effects within the cytoplasm of the cell, often involving phosphorylation or dephosphorylation of various other cytoplasmic proteins, changes in ion channel permeability, or increased concentrations of intracellular molecules which may act as secondary messengers. The important second messengers include cAMP, cytosolic calcium and its associated phosphodiesterase, calmodulin as well as inositol triphosphate and diacylglycerol (see for detail Chapter 6). Some hormones (e.g. insulin and certain growth factors) act at enzymatic receptors in the plasma membrane and then the activated enzyme (e.g. tyrosine kinase) phosphorylates its substrates. The steroid hormones, on the other hand, are able to interact with receptors within the cell because of their ability to penetrate the lipoprotein plasma membrane. The interaction of steroid hormone and receptor forms a complex that in turn combines with specific sites on the chromatin in the nucleus. This results in production of mRNA and subsequent synthesis of new protein through ribosomes that produces the desired biological response (Figure 48.3).

The biological response to a protein or peptide hormone-receptor interaction is more rapid than that to steroid hormone because pre-existing enzymes are activated in former case, whereas the biological response in later requires synthesis of new enzymes and proteins. The combination of the hormone and the receptor usually initiates a cascade of reactions in the cell, so that even a small initiating hormonal stimulus leads to a large final effect. Therefore, the quantitative amounts of hormones those are required to control most metabolic and endocrine functions are very small (few picograms to few micrograms per millilitre of blood). Similarly, the rates of secretion of various hormones are extremely small, usually measured in micrograms or milligrams per day. The most important feedback for hormones is the negative feedback system in which increased hormone concentrations result in less production of the hormones, and vice-versa. Positive feedback systems also exist, although they are much less common than negative feedback systems.

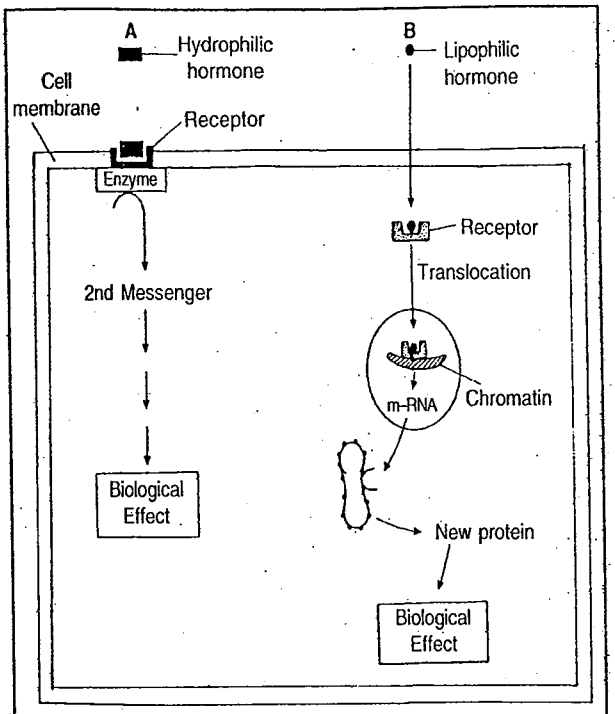


Fig. 48.3. Schematic representation of mechanism of action of hormones (See text for detail).
A: Action of a hydrophilic (protein/peptide or catecholamine) hormone via a membrane receptor.
B: Action of a lipophilic (steroid) hormone via an intracellular receptor.
Abbreviations: m-RNA = messenger ribonucleic acid.

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CHAPTER 49

PITUITARY HORMONES

- Hormones of pituitary gland
- Anterior pituitary hormones
  - Chemistry and classification
  - Growth hormone
    - Growth hormone
    - Growth hormone regulating hormones/factors
      - Growth hormone releasing hormone
      - Growth hormone release inhibitory hormone
    - Insulin-like growth factor-1
    - Disorders of growth hormone
      - Deficiency of growth hormone
      - Excess of growth hormone
    - Treatment of growth hormone disorders
      - Classification
      - Drugs used in deficiency of growth hormone
        - Growth hormone and analogues
        - Growth hormone releasing hormone and analogues
        - Insulin-like growth factor-1 and analogues
        - Drugs used in diagnosis of growth hormone deficiency
      - Drugs used in excess of growth hormone
        - Synthetic analogues of somatostatin
        - Growth hormone receptor antagonists
  - Prolactin
    - Disorders of prolactin secretion
      - Excess of prolactin
      - Deficiency of prolactin
    - Treatment of prolactin disorders
      - Classification
      - Drugs used in excess of prolactin
        - Dopamine agonists
        - Drugs used in deficiency of prolactin
  - Thyroid stimulating hormone
    - Thyroid stimulating hormone regulating hormones
    - Disorders of TSH release
      - Deficiency of TSH
      - Excess of TSH
    - Treatment of TSH disorders
      - Drugs used in deficiency of TSH
  - Gonadotropic hormones
    - Pituitary gonadotropins
    - Gonadotropins releasing hormone
    - Non-pituitary gonadotropins
    - Disorders of gonadotropins
      - Deficiency of gonadotropins
      - Excess of gonadotropins
    - Treatment of gonadotropins disorders
      - Classification
      - Drugs used in gonadotropins deficiency
        - Gonadotropins and analogues
        - Pituitary gonadotropins
        - Non-pituitary gonadotropins
        - Gonadotropin-releasing hormone and analogues
        - Gonadotropin releasing hormone-receptor agonists
      - Drugs used in excess of gonadotropins
        - Gonadoptropin releasing hormone receptor antagonists
        - Synthetic gonadal steroids
  - Adrenocorticotropic hormone
    - Adrenocorticotropic hormone-regulating hormone
    - Disorders of ACTH
      - Deficiency of ACTH
      - Excess of ACTH
    - Treatment of ACTH disorders
      - Classification
      - Drugs used in deficiency of ACTH
        - Adrenocorticotropic hormone and analogues
      - Drugs used in excess of ACTH
        - Corticotropin releasing hormone receptor antagonists
        - Dopamine agonists
        - Mono-amine oxidase inhibitors
        - Antihistamines
- Posterior pituitary hormones
  - Vasopressin/Antidiuretic hormone
    - Disorders of vasopressin
      - Deficiency of vasopressin
      - Excess of vasopressin
    - Treatment of vasopressin disorders
      - Classification
      - Drugs used in deficiency of vasopressin
        - Vasopressin and analogous
        - Miscellaneous drugs
      - Drugs used in excess of vasopressin
        - Vasopressin receptor antagonists /Vaptans
          - Non-selective vasopressin receptor antagonists
          - Selective V1-vasopressin receptor antagonists
          - Selective V2-vasopressin receptor antagonists
        - Tetracyclines
  - Oxytocin
    - Disorders of oxytocin
    - Treatment of oxytocin disorders
      - Classification
      - Drugs used in deficiency of oxytocin
        - Oxytocin and analogues
      - Drugs used in excess of oxytocin
        - Oxytocin receptor antagonists

The pituitary gland (Hypophysis, hypophysis cerebri) is a pea-sized endocrine gland that is attached by a stalk to underside of the cerebrum of the brain. It is often called the 'Master endocrine gland' because it produces a range of hormones and regulates the secretion of many other endocrine glands in the body. It also controls the autonomic nervous system, to some extent. The pituitary gland is itself controlled by the hypothalamus through release of various tropic hormones or factors.

Pituitary gland is composed of two distinct portions arising from two different embryological sites: the anterior pituitary or adenohypophysis and the posterior pituitary or neurohypophysis. Between these parts is a small relatively avascular zone called the pars intermedia, which is particularly well developed in pituitary glands of some species like horses and cattle. Both main parts of pituitary gland have an intimate functional relationship with the hypothalamus, the neurons of which consist of two distinct systems influencing anterior pituitary and posterior pituitary, respectively.

Hormones of Pituitary Gland

The anterior pituitary gland secretes six important hormones viz. growth hormone, prolactin, thyroid stimulating hormone, adrenocorticotropic hormone, follicle stimulating hormone and luteinising hormone and the posterior pituitary gland secretes two important hormones viz. vasopressin and prolactin (Figure 49.1). Special types of secretory cell located in the anterior pituitary lobe synthesise anterior pituitary hormones. The posterior pituitary hormones are synthesised in cell bodies within the hypothalamus and are carried by axon flow to the posterior pituitary, where they are released. Secretions from anterior pituitary are controlled by hormones called hypothalamic releasing and inhibitory hormones or factors, which are secreted within the hypothalamus itself and then conducted to the anterior pituitary. In contrast, secretions from the posterior pituitary are controlled by nerve signal that originates in the hypothalamus and terminates in the posterior pituitary. The hypothalamus in turn receives signals from almost all possible sources in nervous system. The hormones of anterior pituitary play important roles in the control of metabolic functions throughout the body tissues, whereas vasopressin and oxytocin released by posterior pituitary regulate mainly rate of water excretion and milk secretion, respectively.

ANTERIOR PITUITARY HORMONES

The anterior pituitary (Adenohypophysis) synthesises and secretes six major hormones – growth hormone (GH), prolactin, thyroid stimulating hormone (TSH), adrenocorticotropic hormone

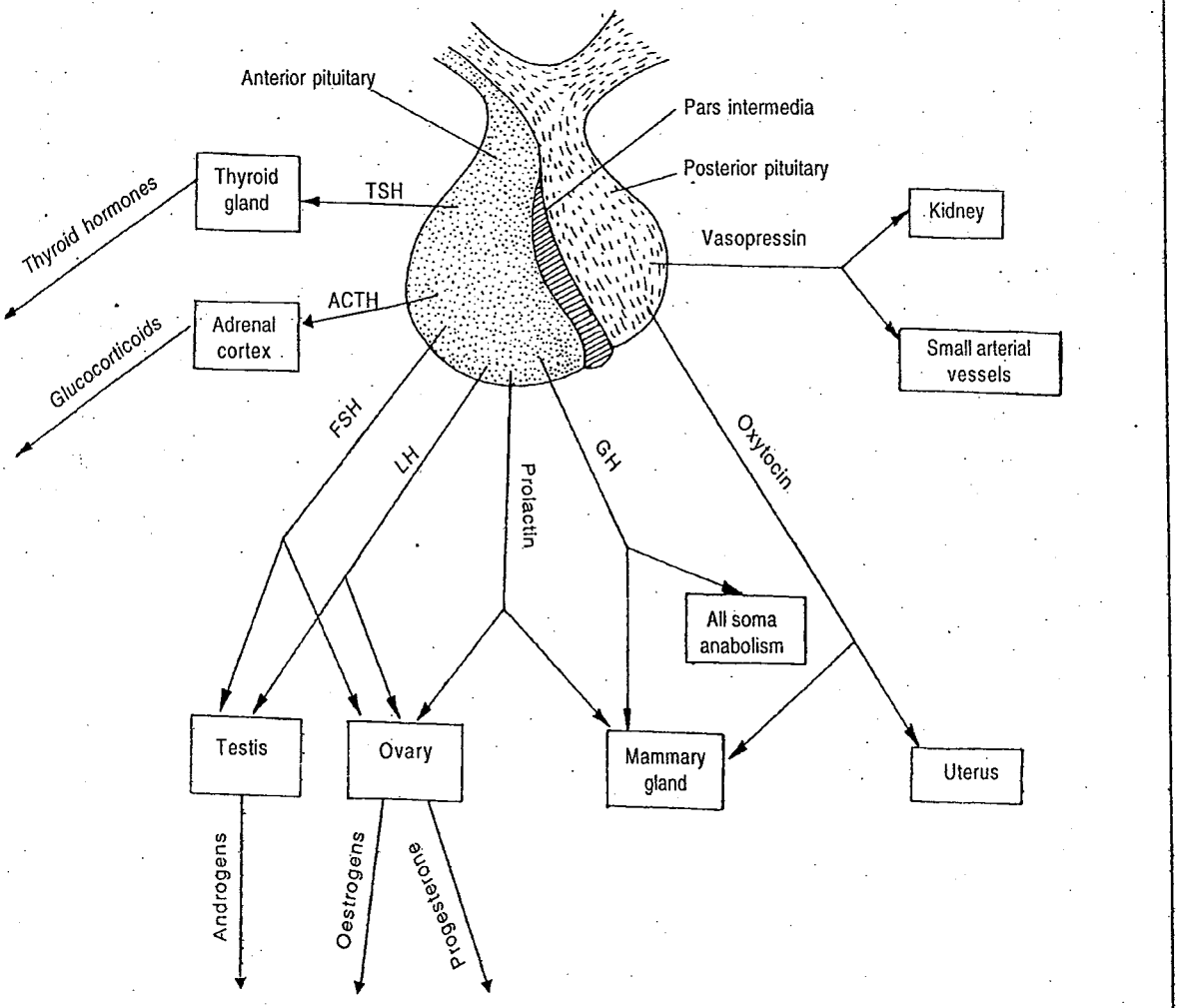


Fig. 49.1. Schematic representation of secretion and action of pituitary hormones.
Abbreviations : GH = growth hormone ; TSH = thyroid stimulating hormone ; ACTH = adrenocorticotropic hormone ; FSH = follicle stimulating hormone ; LH = luteinising hormone.

Table 49.1: Important features of anterior pituitary hormones.

Hormone	Secretary cells	Hypothalamic regulating hormone/factor	Main target site	Important effects/ functions
I. Somatomammotropins				
Growth hormone (GH)	Somatotrophs	Growth hormone releasing hormone (GHRH). Growth hormone release inhibitory hormone (GHRIH, Somatostatin).	General soma	Causes growth of almost all cells and tissues of body; increases protein synthesis.
Prolactin	Lactotrophs	Prolactin release inhibitory factor (PRIF, dopamine).	Mammary gland	Promotes development of mammary tissue during pregnancy, and stimulates milk production in post-partum period.
II. Glycoproteins				
Thyroid stimulating hormone (TSH)	Thyrotrophs	Thyrotropin releasing hormone (TRH).	Thyroid gland	Stimulates synthesis and secretion of thyroid hormones; maintains integrity of thyroid gland.
Follicle stimulating hormone (FSH)	Gonadotrophs	Gonadotropin releasing hormone (GnRH).	Ovary, testis	Causes growth of follicles in ovaries; promotes formation of sperms in testes.
Luteinising hormone (LH)	Gonadotrophs	Gonadotropin releasing hormone (GnRH).	Ovary, testis	Stimulates ovulation and development of corpus luteum in females; regulates testosterone secretion in males.
III. Pro-opiomelanocortins				
Adrenocorticotropin hormone (ACTH)	Corticotrophs	Corticotropin releasing hormone (CRH).	Adrenal cortex	Stimulates secretion of adrenocortical hormones; maintains integrity of adrenal cortex.

(ACTH), follicle stimulating hormone (FSH) and luteinising hormone (LH). All of these are polypeptide in nature and act at extracellular receptors located on their target cells. The growth hormone and prolactin produce their effect mainly directly, whereas other four stimulate the secretory activity of other endocrine glands. Their synthesis and secretion are controlled by the hypothalamus through various releasing and release-inhibiting hormones or factors (Table 49.1) and also by hormones of peripheral endocrine glands via feedback mechanisms.

Chemistry and Classification

The anterior pituitary hormones are produced by separate groups of cells, which are often subdivided into chromophils (acidophils or basophils) and chromophobes based on the interaction of secretory granules with pH-dependent histochemical stains. Growth hormone and prolactin are produced by acidophilic somatotrophs and lactotrophs, respectively and are classified as somatomammotropins (somatotropic hormones); both are single-chain proteins which contain few disulphide bonds. Thyroid stimulating hormone produced by basophilic thyrotrophs and follicle stimulating and luteinising hormones produced by basophilic gonadotrophs are classified as glycoproteins because all three contain carbohydrate moieties. Chromophobes include the corticotroph cells, which belong to the pro-opio-melanocortin and synthesise adrenocorticotropic hormone (ACTH). The corticotroph cells in addition to ACTH produce two melanocyte stimulating hormones (α-MSH and β-MSH) and two lipotropins (β-LPH and γ-LPH), but these are probably not important in mammals. In dogs, pars intermedia contains two population of cells, one of which (corticotroph) can also synthesise adrenocorticotropic hormone (ACTH).

GROWTH HORMONE

Growth Hormone

Growth hormone (Somatotropin, somatotrophin, somatotropic hormone, somatotrophic hormone, STH, GH) is a protein based peptide hormone. It is derived from the somatotroph (somatotrope) cells of anterior pituitary. It is found in the anterior pituitary in larger quantity than any other pituitary hormone. Growth hormone stimulates growth, cell reproduction and regeneration in humans and other animals. It acts either directly on target tissues or indirectly through insulin-like growth factors produced mainly in the liver.

Chemistry

The growth hormone (GH) is a complex protein of molecular weight around 22,000 in most species. In man, GH contains 191

amino acids arranged in a single chain and two disulphide bonds. The complete protein molecule is not necessary for GH action, as fragments of 38 to 40 amino acids have been found to be biologically active. There is considerable species specificity for GH. The human responds only to human or monkey GH, whereas rat responds to most GH. Most domestic animals respond best to homologues GH and less to heterologous GH.

Regulation of secretion

Secretion of growth hormone is regulated by the growth hormone releasing hormone (GHRH) that stimulates growth hormone release and by growth hormone release inhibitory hormone (GHRIH) or somatostatin that inhibits GH release. These factors or hormones bind to their cognate receptors in the somatotrophs and exert their effect through G-proteins mediated increase (GHRH) or decrease (GHIRH) in cytosolic cAMP and intracellular Ca^{++}. Several neurotransmitters, drugs, endogenous substances and other stimuli affect GH secretion by acting on hypothalamus and influencing the release of GHRH or somatostatin. Dopamine, serotonin, alpha-adrenoceptor agonists, fasting, hypoglycaemia, exercise, stress, emotional excitement, sleep, protein rich diet, etc. increase secretions of GH, whereas beta-adrenoceptor agonists, free fatty acids, high doses of glucocorticoids, etc. inhibit GH release. Low blood glucose is believed to be the primary cause of GHRH release, which in turn causes GH secretion. Insulin like growth factor-1 (IGF-1), that is released from liver, and GH itself cause feedback inhibition of GH secretion (Figure 49.2). The GHRIH is also produced by D cells of islets of Langerhans in the pancreas and by some other tissues.

Growth hormone release, like other anterior pituitary hormones secretion, is pulsatile and its plasma concentration fluctuates 10-100 fold. Secretion of growth hormone is high in young and newborns, reaches maximum level during puberty and then decreases to its lowest level during adulthood. Although skeletal growth ceases after puberty, GH has a biological role throughout the life.

Mechanism of action

Growth hormone produces its effects by binding to a specific cell surface enzymatic receptor called growth hormone receptor (GHR), which is widely distributed throughout the body. Binding of growth hormone to the receptor leads to receptor dimerization and the activation of intra- and intercellular signal transduction pathways. Activation of GH receptor has been associated with an increase in the phosphorylation of intracellular proteins mediated by certain cytoplasmic protein kinases. Although GH has direct effects on lipid and carbohydrate metabolism, its anabolic and growth promoting effects are mediated indirectly by other polypeptide hormones collectively called somatomedins or insulin-like growth factors (IGFs). At least four somatomedins have been isolated out of which somatomedin C or IGF-1 is more growth hormone dependent and appears to function as the main mediator of the action of growth hormone because its concentrations in plasma normally follow closely the rate of secretion of growth hormone. The IGF-1 is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion.

The IGF-1 receptors are enzymatic receptors (Tyrosine kinase receptor) present in all tissues and are structurally similar to the insulin receptors. The IGF-1 after binding to its receptor stimulates

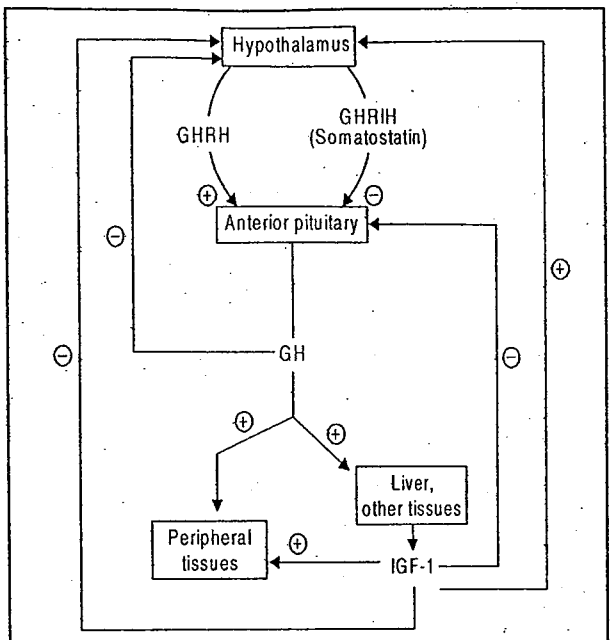


Fig. 49.2. Schematic representation of secretion, regulation and functions of growth hormone (See text for detail).
Abbreviations : GH = growth hormone ; GHRH = growth hormone releasing hormone ; GHRIH = growth hormone release inhibitory hormone ; IGF = insulin-like growth factor, + = stimulation ; – = inhibition.

systemic body growth with growth-promoting effects on almost every cell in the body. These include skeletal muscle, cartilage, bone, liver, kidney, nerves, skin, hematopoietic cell and lungs. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis. Production of IGF-1 is stimulated by growth hormone and can be retarded by undernutrition, growth hormone insensitivity and lack of growth hormone receptors.

Physiological effects

The main pre-pubertal effect of GH is to stimulate normal growth often in conjunction with other hormones secreted by thyroids, gonads and adrenal cortex. Growth hormone is also released throughout life and has an anabolic effect.

Growth promoting and anabolic effects: Growth hormone promotes growth of all organs (except brain and eyes) by inducing hyperplasia. It increases both the soft and osseous tissues in body. Growth of long bones continues as long-as epiphyseal lines do not close. This results from multiple effects of GH on bone including increased deposition of protein by chondrocytic and osteogenic cells which cause bone growth, increased rate of reproduction of these cells and the specific deposition of new bone. Growth hormone is also necessary for hair growth and for development of elastin fibres in the skin.

The GH favours positive nitrogen balance by retention of nitrogen (decreased excretion of nitrogen) by body and causes deposition of protein mainly by enhancing amino acids transport through the cell membrane and by causing synthesis of new proteins. In addition, it decreases catabolism of proteins and amino acids. When administered to cattle (also sheep and pigs but not poultry), GH increases growth rate, feed conversion efficiency and lean to fat ratio. Response of GH is appreciable at younger age, but is lower in older cattle with more fat deposition. These anabolic and growth promoting effects of GH are mainly

mediated indirectly through IGF-1, which are insulin-like and are inhibited by glucocorticoids.

Metabolic effects: Aside from its general effects in causing growth, GH has many specific metabolic effects. Growth hormone conserves carbohydrate, uses up fat stores and enhances rate of protein synthesis. Although the overall effect of GH on carbohydrate and fat metabolism is complex, in general it causes increase in hepatic glucose output (glycogenolysis), decrease glucose utilisation by cells and increased lipolysis. These metabolic effects are mainly mediated directly and are opposite to the effects of insulin (and IGFs); the effects are potentiated by glucocorticoids. Insulin secretion usually increases in response to hyperglycaemia, but high concentrations of GH have diabetogenic effect due to suppression of insulin action.

Growth hormone has also prolactin-like effect because of its structural resemblance to the prolactin. It causes stimulation of mammary glands and favours lactogenesis, increasing milk yield of dairy cows up to 20 percent.

Growth Hormone Regulating Hormones/Factors

Growth hormone releasing hormone

Growth hormone releasing hormone (GHRH), also known as growth-hormone-releasing factor (GHRF) or somatocrinin, is a peptide with 40-44 amino acids. It is produced in the arcuate nucleus of the hypothalamus and is carried by the hypothalamo-hypophyseal portal system to the anterior pituitary gland where it stimulates growth hormone (GH) secretion. The GHRH produces both a short-term effect and a long-term effect. The short-term effect occurs within minutes after GHRH binds to its receptor causing exocytosis of GH storage vesicle, initiating release of GH into the blood. The long-term effect increases transcription in the nucleus by genes which cause new GH synthesis.

Mechanism of action

Growth hormone releasing hormone binds to a specific cell membrane receptor called Growth hormone releasing hormone receptor (GHRHR). The GHRH receptors belong to the superfamily of G protein-coupled receptors and are present on the outer surface of somatotrophs in anterior pituitary. Binding of GHRH to its receptor activates the G_s G-protein, which stimulates adenylate cyclase enzyme leading to enhanced cAMP level in somatotrophs. The enhanced production of cAMP in turn activates the regulatory subunits of protein kinase A (PKA), allowing increase production of GH. The GHRH binding to its receptor also activates the phospholipase C pathway, producing enhanced production of both diacylglycerol (DAG) and inositol triphosphate (IP_3). The second messenger IP_3, in particular, causes release of intracellular Ca^{2+} from the endoplasmic reticulum storage sites, increasing cytosolic Ca^{2+} concentration with resultant increase in vesicle fusion and release of secretory vesicles containing premade growth hormone. Some Ca^{2+} influx also occurs as a direct action of cAMP, which is distinct from the usual cAMP dependent pathway of activating protein kinase A. In addition, the GHRH is also reported to open Na^+ channels by phosphatidylinositol 4,5-bisphosphate, causing cell depolarization. The resultant change in the intracellular voltage opens a voltage-dependent calcium channel, resulting in vesicle fusion and release of GH. The GHRH release from hypothalamus occurs in a pulsatile manner, stimulating similar pulsatile release of GH.

Growth hormone release inhibitory hormone/Somatostatin

Growth hormone release inhibitory hormone (GHRIH), also called growth hormone-inhibiting hormone (GHIH) or somatostatin, is a peptide of 14 amino acids residues. It is released from neurosecretory nerve terminals of periventricular somatostatin neurons and is carried by the hypothalamo-hypophysial portal circulation to the anterior pituitary where it inhibits GH secretion from somatotroph cells. In addition to being found in hypothalamus, it is present elsewhere in the CNS as well as in the pancreas and gastro-intestinal tract.

Mechanism of action

The GHRIH or somatostatin mediates its effect via interaction with G-protein-coupled somatostatin receptors. The binding of somatostatin to its receptors in the anterior pituitary leads to inhibition of adenylate cyclase and voltage dependent Ca^{++} channels and stimulation of K^+ channels. It does not affect GH synthesis but affects mainly the timing and amplitude of the pulses of its release. The somatostatin neurons respond to high circulating concentrations of growth hormone and somatomedins by increasing the release of somatostatin, so reducing the rate of secretion of growth hormone. Its receptors are fairly widely distributed throughout the body and it may have other biological actions in the body. In GI tract, somatostatin suppresses the release of gastrointestinal hormones gastrin, cholesystokinin (CCK), secretin, motilin and vasoactive intestinal peptide (VIP). In comparison with GHRH, its control over GH release is less.

Insulin-like growth factor-1

Insulin-like growth factor-1 (IGF-1), also known as somatomedin C or mechano growth factor, is a polypeptide having molecular weight of about 7500. Although many tissues can synthesise IGFs, the liver is considered to be the major source of circulating IGF-1. The IGF-1 receptor is structurally and functionally analogues to the insulin receptor. It has been postulated that most, if not all, of the growth effects of GH result from IGF-1 and other IGFs rather than from direct effects of growth hormone on bones and other peripheral tissues. Like insulin, IGF-1 promotes lipogenesis and glucose uptake by muscles. IGF-1 is produced throughout life, but its highest concentration is found during the pubertal growth spurt and lowest concentration is noted in infancy and old age. IGF-1 is important for both the regulation of normal physiology, as well as a number of pathological states.

Mechanism of action

The IGF-1 binds to at least two cell surface receptors: the IGF-1 receptor (IGF1R) and the insulin receptor. Both receptor types belong to the superfamily of tyrosine kinase receptors. The IGF-1 receptor appears to be the "physiological" receptor as is present on many cell types in many tissues and binds IGF-1 at significantly higher affinity than the IGF-1 that is bound to the insulin receptor. Binding to the IGF1R initiates intracellular signalling that causes cell growth and proliferation.

DISORDERS OF GROWTH HORMONE

Disorders involving growth hormone usually result from either GH deficiency or GH excess.

Deficiency of growth hormone

Growth hormone deficiency can occur in dogs as a primary abnormality resulting in pituitary dwarfism (panhypopituitarism)

or as an acquired deficiency leading to growth hormone dependent dermatosis. These may be seen by lack of GH, GHRH or a failure of IGFs generation or action. Generalised deficiency of pituitary secretions (pituitary dwarfism) occurs most frequently in German Shepherd dog, but has also been reported in other breeds of dogs. In the pygmy of Africa (a type of human dwarfs), the rate of secretion of GH is normal but there is a hereditary inability to form IGF-1 in response to the GH.

Clinical signs of GH deficiency include abnormal growth (dwarfism). Hypopituitary dwarfism in pups (mainly seen in German Shepherd) is characterised by growth retardation with stunted short stature and a soft, woolly hair coat with retention of secondary (lanugo) hair that progress to bilaterally symmetrical alopecia and skin depigmentation. Dermatological manifestations in adults (mainly seen in Pomeranians, Chow chows, Poodles and Samoyeds) include bilaterally symmetric alopecia of the trunk and severe hyperpigmentation of skin until it is uniformly brown-black over most parts of the body. Deficiency of GH also causes hyperkeratosis, epidermal atrophy, follicular atrophy and thinning of skin due to defective development of elastin fibres in the skin.

Excess of growth hormone

Excessive secretion of GH, GHRH or IGF-1 may result in acromegaly in both human and animals. Acromegaly is often associated with excessive GH secretion in response to progesterone therapy or spontaneous disease. Somatotroph adenonomas (benign tumour of GH producing cells) account for about 80% of cases of human acromegaly. Clinical signs of acromegaly reflect either anabolic or catabolic abnormalities. Increased body size results from proliferation of bone and connective tissues, particularly of the limbs, feet, head and abdomen. The most common is inspiratory stridor (due to soft tissue accumulation in the thorax). Thickening of skin may be present. In humans, acromegaly is seen in adults after the epiphyseal lines close. This causes thickening of the membranes and long bones and enlargement of facial structures, hands and feet. Before puberty, enhanced GH secretion results in gigantism characterised by increased lengthening of long bones and enhanced deposition of soft tissues.

TREATMENT OF GROWTH HORMONE DISORDERS

A pharmacological approach to growth hormone disorders is an important part of modern medicine. Drugs are available for treatment of both GH deficiency and GH excess.

Classification

Drugs used to treat GH disorders have been broadly classified into two classes: drugs used in deficiency of GH and drugs used in excess of GH.

I. Drugs used in deficiency of GH
   1. Growth hormone and analogues
      e.g. growth hormone (somatotropin), somatropin and somatrem.
   2. Growth hormone releasing hormone and analogues
      e.g. sermorelin.
   3. Insulin-like growth factor-1 and analogues
      e.g. mecasermin.
   4. Drugs used in diagnosis of GH deficiency
   a. Alpha$_2$-adrencoceptor agonists
      e.g. clonidine and xylazine.

II. Drugs used in excess of GH
   1. Synthetic analogues of somatostatin
      e.g. octreotide and lanreotide.
   2. Growth hormone receptor antagonists
      e.g. pegvisomant.
   3. Other drugs
      e.g. bromocriptine.

I. Drugs Used In Deficiency of Growth Hormone

1. Growth Hormone and Analogues

Growth hormone/Somatotropin

Growth hormone (Somatotropin, somatotrophin, ST, GH) is commercially available as human, bovine and porcine growth hormone. In humans, production of GH from cadaver pituitary has been suspended because of suspicion of transmission of a fatal viral illness by it and also because of availability of synthetic hormones. Human and bovine GHs produced by recombinant DNA technique are now available and used widely. Human recombinant GH (somatotropin) has amino acid sequence identical to that found in body. Although the GHs of different species are sufficiently different from one another, human and bovine (also porcine) GHs are biologically active in dogs. Recombinant canine GH is also prepared and is being studied in dogs. At present, recombinant DNA-derived bovine somatotropin, (rbST, rbGH) is most widely used recombinant veterinary product.

Pharmacological effects

The mechanism of action and pharmacological effects produced by exogenous GH is similar to those of natural GH. Exogenous administration of recombinant bovine GH (rbST) increases growth rate, improves feed efficiency and suppresses fat deposition in calves. In lactating animals, rbST increases milk production and extends calving interval.

Pharmacokinetics

Pharmacokinetic literature on GH in animals is not complete. It is not active orally as GH is rapidly metabolised and cleared by gut. In man, maximum plasma concentration of GH is seen after 2-6 hours of IM or SC injection and peak plasma concentration of IGF-1 is apparent after 20 hours. About 20% of the circulating hormone is bound to growth hormone binding protein. Growth hormone is degraded mainly in liver, kidneys and some other tissues. The degradation products (amino acids) are recycled to make new protein in the same way as other endogenous proteins. Growth hormone has half-life of 20-30 minutes, but due to slow induction and clearance of IGF-1, the biological effects of GH outlast its survival.

Side effects/Adverse effects

Important side/adverse effects of exogenous GH include hypersensitivity reaction due to formation of antibodies to it. Subcutaneous injections may lead to local lipoatrophy and mild transient swelling at the injection site. High doses may produce diabetes mellitus and ketosis (due to formation of excessive fat from adipose tissues and formation of ketone bodies). The excessive mobilisation of fat from adipose tissue may also cause a fatty liver. Studies in swine, dogs and cats have indicated that GH injection may lead to induction of persistent hyperglycaemia and damage to cells of islet of Langerhans; pancreatic beta cells initially produce excessive insulin to cope with hyperglycaemia, but soon get exhausted and undergo degeneration.

In cattle, fertility parameters can be impaired with reduced reproductive efficiency by a high dose or early administration of GH after calving. Reduced pregnancy rates, as a result of cystic ovaries and disorders of uterus, are caused by bST. Gestation period becomes shorter with reduced birth weight of offspring and increased rate of twining and placental retention. Clinical and subclinical mastitis may be observed with bST.

Contraindications and precautions

The use of GH preparations should be avoided in food-producing animal. Treatment with GH should be stopped, if glycosuria occurs. Weekly monitoring of blood glucose before and after therapy is recommended. Although GH is reported to be present in the milk of treated animal, it is not considered harmful to human health because the hormone is destroyed by pasteurisation of milk and also because it is digested in the GI tract. The digested fragments are not biologically active.

Drug interactions

Growth hormone has shown interactions with other hormones like glucocorticoids or insulin, so care should be taken when they are used concurrently.

Clinical uses

Growth hormone is not frequently used in veterinary medicine because it is expensive, species specific and has antigenic properties. It can be used clinically in the treatment of pituitary dwarfism and GH-responsive alopecia in dogs. In GH-responsive alopecia, therapy should be discontinued once dermatological signs have reduced. Although the use of bovine GH in livestock production science is till debatable, some veterinarians use it to enhance milk production in cattle and also as a growth promoter in calves. Sustained release (14-28 days) vehicles have been developed for use in cattle to obviate the need of daily injection. If used in cattle for enhancing milk production, growth hormone should be used after about 60 days post-calving with standard management for high yielding cows.

Dose

For GH-responsive alopecia and pituitary dwarfism

Bovine and Human GH

Dogs : 0.1 IU/kg, SC, 3 times weekly for up to 6 weeks.

Porcine GH

Dogs : 2 IU (total), SC, every alternate for 100 days.

To stimulate milk production

Cattle : 25 mg (total), IM, once daily.

: 500 mg (total) of sustained slow release preparation, IM or SC, once every 15 days.

Somatropin

Somatropin is a recombinant human growth hormone (rhGH). It contains the identical amino acid sequence of human GH produced by the pituitary gland. Its pharmacological effects are similar to those of endogenous growth hormone. It is indicated in children for long-term treatment of growth failure caused by pituitary growth hormone (GH) deficiency (pituitary dwarfism), including GH deficiency caused by cranial irradiation. It may also be indicated in adults for treatment of growth failure caused by GH deficiency.

Somatrem

Somatrem, also called met-GH, the human growth hormone (hGH) analogue, is prepared by genetic engineering with an additional methionyl residue. It has the same amino acid sequence as human GH with an extra methionine at the end of the chain to facilitate the manufacturing process. It is equally effective, but more antigenic than naturally occurring somatotropin. It is less used nowadays or has even been discontinued due to serious side effects.

2. Growth Hormone Releasing Hormone and Analogues

Human growth hormone releasing hormone is used mainly as a diagnostic agent to know if the GH deficiency is due to a hypothalamic or a pituitary defect. In human medicine, about 40-80% of GH deficient children respond to GHRH suggesting that the defect in many children is hypothalamic. Provocative testing for the diagnosis of GH deficiency with human GHRH has also been studied in dogs. Administration of human GHRH causes 2-4 fold increase in serum GH in normal dogs compared with no increase in dogs with GH deficiency.

Sermorelin

Sermorelin is a synthetic analogue of GHRH containing the first 29 amino acid residues as found in human GHRH. It is available as a sterile, non-pyrogenic, lyophilized powder intended for subcutaneous injection after reconstitution with sodium chloride solution. It has been introduced in human medicine as a diagnostic test for growth hormone secretion. It is also misused as doping substance in sports. Pain, swelling or redness at the injection site may occur. Although an allergic reaction to sermorelin is unlikely, but immediate medical attention is recommended if it occurs.

3. Insulin-Like Growth Factor-1 and Analogues

Laron-type dwarfism in humans occurs due to mutations of GH receptor gene that either prevents GH binding or causes other defects in the receptor. Such patients have normal levels of GH but decreased levels of IGF-1. Such dwarfs have been treated with recombinant human IGF-1.

Mecasermin

Mecasermin is recombinant human insulin-like growth factor 1 (IGF-1) manufactured by using both yeast and *E. coli*. It is used for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency. Mecasermin is not recommended for use in children who have stunted growth due to growth hormone deficiency, malnutrition, underactive thyroid, or those who are taking long-term steroid medications. Mecasermin is supplied as a sterile, aqueous, clear and colourless solution intended for subcutaneous injection.

4. Drugs Used in Diagnosis of Growth Hormone Deficiency

Although GH assays are available in veterinary medicine, a GH stimulation test is often required for diagnosis of GH deficiency. Changes in GH concentrations in response to stimulation are usually used to diagnose the disorders of GH secretion.

Alpha$_2$-adrenoceptor agonists

Selective alpha$_2$-adrenoceptor stimulants like clonidine or xylazine is often used to stimulate secretion of GH from anterior pituitary. Both drugs induce production of endogenous GHRH thereby stimulating release of the GH. Intravenous administration of xylazine (0.1 mg/kg) or clonidine (0.01 mg/kg) causes increase in circulating GH in normal dogs, whereas dogs with GH deficiency do not respond to the treatment.

II. Drugs Used in Excess of Growth Hormone

1. Synthetic Analogues of Somatostatin

Octreotide

Octreotide acetate is synthetically prepared octapeptide that mimics activities of natural somatostatin. Octreotide binds to the same receptors as somatostatin, although with higher affinity to peripheral receptors. Similar to somatostatin, octreotide inhibits secretion of growth hormone and many other hormones, such as gastrin, glucagon, insulin, secretin, TSH and cholesystokinin, pancreatic polypeptide and vasoactive intestinal peptide. It also reduces secretion of fluids by the intestine and pancreas and inhibits motility of GI tract and contraction of gall bladder. By decreasing secretion of GI fluids, octreotide helps to reduce the loss of body fluids and minerals. It has also been shown to produce analgesic effects, most probably acting as a partial agonist at the mu opioid receptor.

Octreotide is absorbed quickly and completely after subcutaneous application. In humans, maximal plasma concentration is reached after 30 minutes and the drug is eliminated in about 100 minutes. About 32% of a dose is eliminated unchanged in the urine. Important side/adverse effects associated with octreotide use in humans include headache, GI disturbances (e.g. nausea, vomiting, diarrhoea or constipation, and abdominal cramps), cardiac arrhythmias, pruritus, dyspnoea, etc. Rare side effects include acute anaphylactic reactions, pancreatitis and hepatitis. Patients with diabetes mellitus might need less insulin or oral antidiabetics when treated with octreotide.

Octreotide has been approved for the treatment of acromegaly in humans. It has also been used for the treatment of diarrhoea and flushing episodes associated with carcinoid syndrome, and treatment of diarrhoea in patients with vasoactive intestinal peptide-secreting tumours. In dogs, octreotide has been used for treatment of some insulinomas and gastrinomas. Octreotide comes as an immediate-release injection for administration by SC or IV routes and as a long-acting injection for IM use.

Lanreotide

Lanreotide is a synthetic analogue of somatostatin. Similar to octreotide, it inhibits the release of several other hormones, including growth hormone, thyroid stimulating hormone (TSH), insulin and glucagon. Lanreotide is used in the treatment of acromegaly, due to both pituitary and non-pituitary growth hormone-secreting tumours, and the management of symptoms caused by neuroendocrine tumours, particularly carcinoid tumours and vasoactive intestinal peptide adenomas. Important side/adverse effects associated with lanreotide treatment include mild to moderate pain at the injection site and gastrointestinal disturbances, such as diarrhoea, nausea and vomiting. Lanreotide is available commercially in two formulations: a sustained release formulation for intramuscular use and an extended release formulation for subcutaneous use.

2. Growth Hormone Receptor Antagonists

Pegvisomant

Pegvisomant is a growth hormone receptor antagonist used in the treatment of acromegaly. It is basically a genetically modified *Escherichia coli* bacterial protein containing 191 amino acid residues to which several polyethylene glycol polymers have been added. It structurally resembles growth hormone as a result of which it occupies the GH receptor and blocks it. By blocking action of GH pegvisomant inhibits the synthesis of IGF-1 in the liver. It has been recommended for the treatment of pituitary gland tumour causing the acromegaly, especially when the pituitary tumour cannot be controlled with surgery or radiation and the use of somatostatin analogues is unsuccessful. Side effects of pegvisomant include reactions at the injection site, swelling of limbs, chest pain, hypoglycemia, nausea and hepatitis. It also produces rebound GH release because a decrease level of IGF-1 stimulates GH synthesis and secretion due to negative feedback control.

3. Other Drugs

Several other drugs have been tried in the treatment of acromegaly in humans and animals, but none has proven effective in all conditions. Dopamine agonists such as bromocriptine are used occasionally for treatment of acromegaly in some species. In human medicine, bromocriptine is used to suppress the secretion of GH by adenomas (bromocriptine stimulates GH secretion in normal subjects, but suppresses it in adenomas).

PROLACTIN

Prolactin

Prolactin (Lactogenic hormone, luteotropic hormone, mammotropin, luteotropin) is a peptide hormone that is structurally related to growth hormone and belongs to somatomammotropin hormone family. It is secreted by mammotroph (lactotroph) cells which are abundant in the anterior pituitary and increase in number during pregnancy, probably under the influence of oestrogen. Prolactin is involved mainly in the development of mammary gland and maintenance of lactation. It is luteotropic in bitches and queens and also in sheep.

Chemistry

Human and ovine prolactin is a single-polypeptide chain of molecular weight about 23,000, containing three disulphide bridges. Prolactin consists of 198 amino acids in sheep. In humans, dimeric (Mol. wt. 48000-56000) and polymeric (Mol. wt. >100000) forms of prolactin have also been identified, but biological significance of these forms is not known. Because prolactin resembles growth hormone (also placental lactogen) in structure, it is thought that these two polypeptides are evolved from a common ancestral cell.

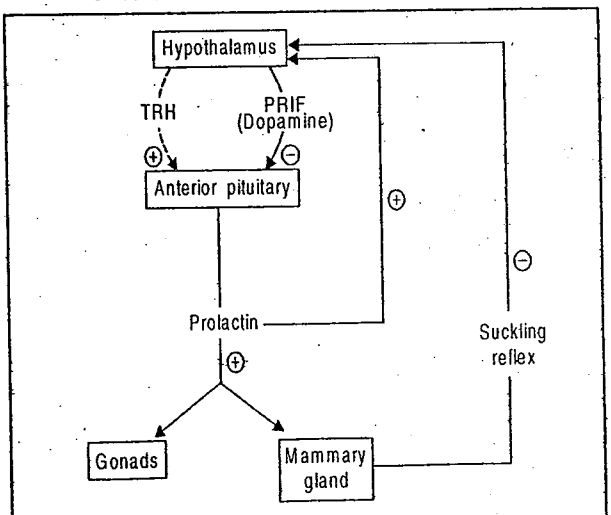


Fig. 49.3. Schematic representation of secretion, regulation and function of prolactin (See text for detail).
Abbreviations : PRIF = prolactin release inhibitory factor ; TRH = thyrotropin releasing hormone, + = stimulation ; − = inhibition.

Regulation of secretion

The anterior pituitary secretion of prolactin is controlled almost entirely by a hypothalamic inhibitory factor called prolactin release inhibitory factor, which is identified as dopamine. The dopamine is produced by neurons in the ventral hypothalamus (arcuate nucleus) and then released into portal capillaries to gain access to lactotrophs, where it binds to the D_2-dopamine receptor to inhibit prolactin secretion. Unlike other anterior pituitary hormones, the secretion of prolactin is not stimulated by any effective hypothalamic hormone or factor. Thyrotropin releasing hormone (TRH) is suspected to be involved in the release of prolactin but its significance is not confirmed. Moreover, unlike other anterior pituitary hormones, prolactin does not stimulate synthesis and secretion of other hormones in its target cells (i.e. does not act indirectly) and is also not influenced by feedback regulation by agents synthesised in the periphery. Rather, prolactin itself is believed to accelerate dopamine turnover and has a negative feedback control over its release (Figure 49.3).

Like GH, prolactin is secreted in a pulsatile manner and its output is increased by sleep, stress, exercise and hypoglycaemia. Prolactin concentration rises markedly during pregnancy, reaching its maximum at term. In lactating animals, prolactin secretion is stimulated by the suckling stimulus or milking, which sends sensory stimulus to the hypothalamus wherein synthesis of dopamine is blocked. A short-lived surge of prolactin secretion occurs immediately after the onset of milk removal and its level rises to 10-100 fold within 30 minutes of initial stimulus. Prolactin secretion decreases as lactation period progresses and declines after several months of lactation. Drugs which block dopamine receptors (e.g. phenothiazine tranquillizers and butyrophenones) also increase prolactin secretion and cause an increase in milk production. Vasoactive intestinal peptide (VIP) inhibits dopamine synthesis within the hypothalamus, whereas oestrogen decreases lactotrophs sensitivity to dopamine; both are stimulators of prolactin secretion. Dopamine and dopamine agonists (e.g. bromocriptine), on the other hand, cause reduced synthesis and output of prolactin.

Mechanism of action

The prolactin mediates its effect through prolactin receptors, which are structurally related to the GH receptors. The prolactin receptor is an enzymatic cytokine receptor, with an extracellular region that binds prolactin, a transmembrane region and an intracellular cytoplasmatic region. The second messenger cascade is the JAK-STAT pathway (JAK is short for 'Janus kinase or just another kinase', and STAT is short for 'signal transducer and activator of transcription'). The JAK-STAT signalling pathway is involved mainly in the mechanisms of interferons, interleukins, growth factors, hormones or other chemical messengers, which regulate DNA transcription and activity in the cell. The JAK-STAT system consists of three main components: a membrane bound receptor, a JAK (a family of intracellular, non-receptor tyrosine kinases) and STAT (a family of intracellular proteins which regulate some aspects of growth, survival and differentiation in cells). When the ligands such as prolactin bind to the receptor, the information is transmitted through the cell membrane to activate the tyrosine kinase activity of JAK, which autophosphorylates itself. The STAT protein then binds to the phosphorylated receptor and gets itself phosphorylated. The phosphorylated STAT protein then translocates into the cell nucleus, where it binds to DNA and promotes transcription of genes responsive to STAT.

Similar to prolactin, the growth hormone can also bind to prolactin receptors and thus has prolactin like lactogenic effects. Contrary to this, prolactin cannot bind to GH receptor so does not have somatotropic activity. Unlike GH, all actions of prolactin are mediated directly i.e. it does not have any intermediary hormone.

Physiological effects

The main physiological function of prolactin in females is control of milk production (i.e. lactogenesis). Prolactin, in a complex interaction with other circulating hormones (e.g. growth hormone, gonadotropins, oestrogens, progesterons, thyroid hormones and adrenocortical hormones), prepares the mammary gland for post-partum lactation. During pregnancy, high levels of oestrogen and progesterone inhibit prolactin activity, but at parturition when blood levels of oestrogen fall the prolactin concentration rises and lactation is initiated. Prolactin stimulates RNA synthesis for milk production proteins and promotes lactose genesis.

Prolactin exerts an anti-gonadotropic effect by inhibiting release and/or response of ovaries to the gonadotropins. Therefore, the elevated prolactin levels in lactating females in some species result in suppression of ovulation. As a result pathological hyperprolactinaemia is a common cause of infertility in females. Prolactin also possesses luteotropic effect in bitches and queens and is suspected to be involved in the maintenance of anoestrous phase of the oestrous cycle in bitches. The decline of progesterone and increase in prolactin production at the termination of dioestrus cause the clinical manifestation of pseudopregnancy in the non-pregnant bitches. Non-pregnant bitches can nest, lactate and nurture objects during this time. There is a diurnal as well as an ovulatory cycle in prolactin secretion. In many mammals, there is also a seasonal change in prolactin release.

In addition to their presence in the mammary gland, prolactin receptors are present in many tissues including ovaries, pituitary glands, heart, lung, thymus, spleen, liver, pancreas, kidney, adrenal gland, uterus, skeletal muscle, skin and areas of the central nervous system. The physiological effects of prolactin at these sites are poorly characterised. Prolactin receptors also are present in T lymphocytes and prolactin has been shown to modulate the immune response.

DISORDERS OF PROLACTIN SECRETION

Excess of Prolactin

Hyperprolactinaemia is a common syndrome in humans and animals. Hyperprolactinaemia may be caused by prolactin secreting tumours, disorders of hypothalamus or pituitary affecting prolactin secretion (mainly failure of dopamine at dopamine receptor levels in the anterior pituitary or a reduction of its synthesis in the hypothalamus), drugs like dopamine antagonists, primary hypothyroidism associated with increased TRH levels, etc. Clinical manifestations of hyperprolactinaemia may include galactorrhoea and infertility in females. As prolactin secretion alters ovarian responsiveness to gonadotropins, continued presence of prolactin during anoestrous phase may prolong duration of this phase of the oestrous cycle in bitches.

Antiprolactin drugs (e.g. dopamine agonists) are used clinically to control pathological hyperprolactinaemia in various species and also to shorten the naturally occurring long interoestrous interval in bitches.

Deficiency of Prolactin

Deficiency of prolactin can cause suppressed mammary development and improper lactation. Suppressed mammary development is a feature of chronic ergot poisoning in ewes.

TREATMENT OF PROLACTIN DISORDERS

A pharmacological approach to prolactin disorders is a valuable part of veterinary and human medicine. Drugs are available mainly for treatment of hyperprolactinaemia.

Classification

Drugs used to treat prolactin disorders have been broadly classified into two classes: Drugs used in excess of prolactin and drugs used in deficiency of prolactin.

I. Drugs used in excess of prolactin
   1. Dopamine agonists
      e.g. bromocriptine, cabergoline and metergoline.
II. Drugs used in deficiency of prolactin
   e.g. growth hormone and placental lactogen.

I. Drugs Used in Excess of Prolactin

1. Dopamine Agonists

Bromocriptine

Bromocriptine (Bromocryptine, brom-ergocryptine) is a semi-synthetic ergot derivative, which is used as a dopamine agonist and prolactin inhibitor. It is used in the treatment of pseudopregnancy and pituitary adenomas. However, its use has not gained wide acceptance because of its adverse effects.

Mechanism of action

Bromocriptine is a potent dopamine agonist. It directly activates dopamine receptors present on the lactotroph cells and inhibits the secretion of prolactin. Bromocriptine has greater action on D_2 receptors in the CNS; at certain dopamine sites in the brain it acts as a partial agonist or antagonist at D_1 receptor. Bromocriptine also inhibits the release of glutamate, by reversing the glutamate GLT-1 transporter. It has also a weak adrenoceptor blocking action.

Pharmacological effects

Bromocriptine exhibits multiple pharmacological actions. It inhibits prolactin release from the anterior pituitary thereby reducing serum prolactin level. Hence it has an antigalactopoietic effect. Inhibition of prolactin secretion can be luteolytic during the second half of dioestrus or pregnancy in bitches and queens. Decrease in prolactin secretion can shorten the length of anoestrus and can induce oestrus if treatment occurs during late anoestrus.

Bromocriptine increases growth hormone release in normal subjects but decreases the same from pituitary tumour. It produces nausea and vomiting through stimulation of dopaminergic receptors in chemoreceptor trigger zone. Bromocriptine may also produce hypotension due to weak peripheral alpha-adrenoceptor blockade and through central effects. Bromocriptine may decrease the secretion of corticotropin in some animals with pituitary dependent hyperadrenocorticism.

Pharmacokinetics

Pharmacokinetics data on bromocriptine in domestic animals are scarce. In humans, about 1/3 of an oral dose of bromocriptine is absorbed from gut but due to a high first-pass effect only about 6% reaches systemic circulation. It is highly bound to plasma proteins (>90%). It is metabolised by the liver. It has a biphasic half-life, the initial alpha phase is about 4 hours and the terminal phase is about 15 hours.

Side effects/Adverse effects

The side/adverse effects of bromocriptine are frequent and dose-related. Important adverse effects include nausea, vomiting, anorexia, sedation, fatigue, hypotension and behavioural changes. Bromocriptine is often associated with protracted vomiting, which limits its usefulness.

Treatment: Treatment of bromocriptine overdosage is symptomatic and supportive. Cardiovascular support should be instituted as needed.

Contraindications and precautions

Bromocriptine is contraindicated during pregnancy and hepatic insufficiency. It should not be used in lactating animals as it interferes with lactation. It should be used carefully in patients having cardiovascular problems.

Drug interactions

Bromocriptine should not be used along with butyrophenones, phenothiazines, reserpine or other antidopaminergic drugs because they may increase prolactin concentration. Oestrogen and progesterone may also interfere with the effects of bromocriptine. Concurrent use of antihypertensive drugs may produce additive hypotensive effect.

Clinical uses

In veterinary medicine, bromocriptine is used mainly for shortening anoestrus and in pseudopregnancy in bitches. It is also useful in treating acromegaly or pituitary adenomas in a variety of species. Bromocriptine is used in the treatment of pituitary dependent hyperadrenocorticism in dogs, but due to adverse effects its clinical value is low. In humans, bromocriptine is considered the drug of choice for treatment of prolactinaemia regardless of the aetiology or the sex of patient. Bromocriptine has also been used to treat syndromes of growth hormone excess and in treatment of Parkinsonism often as an adjunct to levodopa.

Currently, bromocriptine is less used in veterinary medicine because of its potential adverse effects and availability of less toxic newer drugs (e.g. cabergoline). Bromocriptine should be reserved for cases where other methods fail. Long duration of treatment (i.e. 40 days) is required to induce oestrus in bitches.

Dose

For treatment of pseudopregnancy

Dogs : 0.01 mg/kg, PO, 2 times daily for 10 days.
: 0.03 mg/kg, PO, once daily for 16 days.

For treatment of hyperadrenocorticism

Dogs : Up to 0.1 mg/kg, PO, in divided doses in gradually increasing amount.

Horses : 0.1 mg/kg, PO, 2 times daily.

Note: If vomiting occurs in dogs as a result of bromocriptine therapy, metoclopramide may be used although it is a dopamine antagonist.

Cabergoline

Cabergoline is a potent D_2-dopamine receptor agonist, although low affinity interactions with D_1-dopamine receptors, α_1-adrenergic-receptors and α_2-adrenergic-receptors have also been noted. Cabergoline has recently been introduced in veterinary

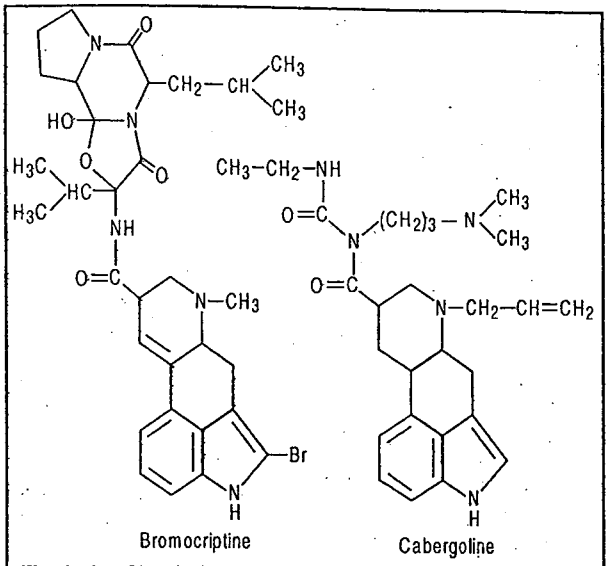


Fig. 49.4. Chemical structures of selected dopamine agonists used in excess of prolactin.

medicine for treatment of pseudopregnancy and suppression of lactation in bitches. It is also used occasionally for termination of pregnancy. Cabergoline is occasionally used as an adjunctive or second line therapy of acromegaly, although its efficacy in suppressing growth hormone levels is much lower than that suppressing hyperprolactinaemia induced by prolactin.

Cabergoline has fewer side effects, which include transient hypotension, transient drowsiness and occasionally vomiting or anorexia. Side effects are mostly dose dependent. Following a single oral dose, absorption of cabergoline from the gastrointestinal (GI) tract is highly variable, typically occurring within 0.5 to 4 hours. Cabergoline is expensive that limits its routine use in animals.

Dose

Dogs : 0.05 mg/kg, PO, once daily for 4 to 6 days. It may be given mixed with food.

Metergoline

Metergoline, an ergot derivative, is a dopamine agonist with actions and uses similar to those of bromocriptine. It is also a serotonin antagonist. Metergoline has been used similar to bromocriptine in disorders associated with hyperprolactinaemia. It is used mainly to suppress lactation, to shorten length of anoestrus (i.e. to induce oestrus) and for treatment of pseudopregnancy in bitches. In human medicine, it also used for the treatment of gastrointestinal disorders and for the prophylaxis of migraine and other vascular headaches. Its actions are similar to bromocriptine but have no apparent side effect.

Dose

For induction of oestrus

Bitches : 12.5 mg (total), IM, every 3 days until the onset of pro-oestrus. Induction of oestrus may occur within 40 days of the start of therapy.

II. Drugs Used in Deficiency of Prolactin

Prolactin itself has no therapeutic value. In veterinary medicine, no specific drug is used to enhance prolactin concentration in body. Growth hormone is used occasionally in lactating cows to promote milk production. In human medicine, use of placental lactogen (a glycoprotein synthesised by placenta) is suggested occasionally to complement actions of prolactin in the growth and development of breast.

THYROID STIMULATING HORMONE

Thyroid Stimulating Hormone

Thyroid stimulating hormone (Thyrotropic hormone, thyrotropin, thyrotrophin, TSH) is a glycoprotein hormone, synthesised and secreted by thyrotroph cells of the anterior pituitary. It regulates the activity of thyroid gland, including the synthesis and release of thyroid hormones.

Chemistry

Thyroid stimulating hormone is a glycoprotein with a molecular weight of about 30,000. There is considerable species specificity to the hormone. In man, TSH contains two glycoprotein chains having 210 amino acids and about 22% sugar.

Regulation of secretion

Secretion of TSH from the anterior pituitary is controlled by the thyrotropin-releasing hormone (TRH), a hypothalamic hormone. The negative feedback inhibiting TSH secretion is provided by the thyroid hormones, which act primarily at the level of pituitary (Figure 49.5). Triiodothyronine (T_3) has been shown to reduce TRH receptors on thyrotrophs. Exposure to cold and emotional reactions increase the output of TRH and thereby TSH, whereas excitement and anxiety or other conditions which greatly stimulate the sympathetic nervous system decrease secretion of TSH. Glucocorticoids inhibit secretion of TSH at both the hypothalamus and pituitary gland.

Mechanism of action

Thyroid stimulating hormone mediates its effect through the TSH receptors present on follicular cells of the thyroid gland. The TSH receptor is a member of the G protein-coupled receptor superfamily and is coupled to the G_s protein. The TSH receptors utilise the adenylate cyclase-cAMP transducer mechanism to produce their effects. In addition, high concentration of TSH may

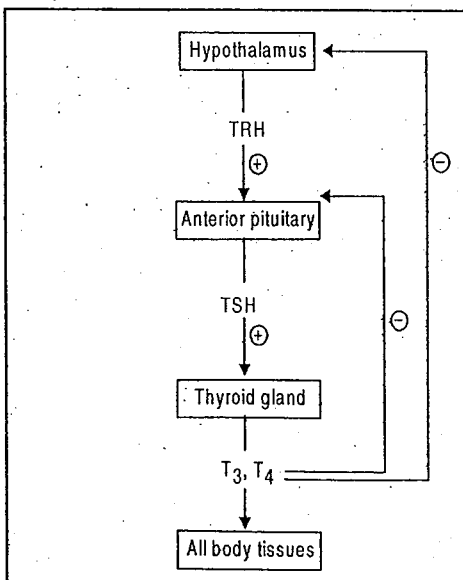


Fig. 49.5. Schematic representation of secretion and regulation of thyroid stimulating hormone (see text for detail).
Abbreviations : TRH = thyrotropin releasing hormone ; TSH = thyroid stimulating hormone/thyrotropin ; T_3 = triiodothyronine ; T_4 = thyroxine, + = stimulation ; − = inhibition.

ıuse increase in cytosolic Ca^{++} and protein kinase C activation rough phosphatidyl inositol-4,5-bisphosphate (PIP_2) hydrolysis thyroid cells, which may also be involved in TSH actions.

hysiological effects

Thyroid stimulating hormone increases synthetic and cretory activity of the follicular cells of thyroid gland. Thyroid imulating hormone induces hyperplasia and hypertrophy of yroid follicles and increases blood supply to the gland. It :tivates the iodine pump that increases rate of 'iodide trapping' the glandular cells. This increases iodination of tyrosine and ıupling to form the thyroid hormones. Thyroid stimulating ɔrmone increases proteolysis of thyroglobulin that has already :en stored in the follicles, with the resultant release of thyroid ɔrmones into circulating blood (see for detail Chapter 50). The ·oteolytic effect on thyroglobulin that causes release of T_3 and $_4$ thyroid hormones occurs early (within 30 minutes), whereas her effects require hours or even days and weeks to develop.

hyroid Stimulating Hormone Regulating Hormones

hyrotropin-releasing hormone

Thyrotropin releasing hormone (TRH) is a tripeptide amide ɔyroglutamyl-histidyl-proline amide), which causes release of TSH lso prolactin) from the anterior pituitary. It is manufactured in .e hypothalamus and transported to anterior pituitary gland via .e superior hypophyseal artery. In addition to endocrine function, RH has neurotransmitter and paracrine function as well. It imulates synthesis and release of TSH from anterior pituitary by inding to the TRH receptors in the thyrotroph cell membrane. his in turn activates the phospholipase second messenger system ı produce large amounts of phospholipase C, followed by many :her second messenger products including calcium ions and iacylglycerol, which eventually lead to TSH release.

ISORDERS OF TSH RELEASE

eficiency of TSH

Deficiency of TSH is involved only in a few cases of ypothyroidism. Deficiency of TSH secretion from the pituitary land results in secondary hypothyroidism, whereas lack of ypothalamic TRH and its subsequent effects of TSH results in ɔrtiary hypothyroidism. Long-term thyroid stimulation by an nmunoglobulin of IgG class in Grave's disease in humans often ɔsults in low TSH levels (excessive thyroid secretion suppresses 'SH production via the negative feedback mechanism). In dogs, ɔcondary hypothyroidism occurs mainly due to destruction of ituitary thyrotrophs by an expanding large tumour and in some ases also with congenital hypopituitarism (pituitary dwarfism).

The deficiency of TSH results in impaired production and ɔcretion of the thyroid hormones. The clinical signs of secondary r tertiary hypothyroidism resemble those of primary ypothyroidism and refer to multiple body systems (see for detail 'hapter 50).

:xcess of TSH

Excess of TSH may be encountered in thyroid gland ısufficiency, where low thyroid gland secretions stimulate the ynthesis of TRH at level of hypothalamus and by activation of 'SH at level of pituitary. Excess of circulating TSH is possibly ɔsponsible for the increase in size of thyroid that occurs in ɔdine deficiency and other goitrogenic circumstances. Enhanced ıyroid activity due to excess of TSH or TRH is not encountered clinically. Hyperthyroidism resulting in excessive secretion of thyroid gland primarily occurs due to defects (e.g. neoplasia) in thyroid gland itself.

TREATMENT OF TSH DISORDERS

I. Drugs Used in Deficiency of TSH

Thyroid stimulating hormone

Thyroid stimulating hormone (TSH) or thyrotropin is obtained commercially from the bovine anterior pituitary gland. Commercial TSH is used primarily as a diagnostic agent to differentiate between primary hypothyroidism and secondary hypothyroidism due to pituitary failure. On TSH administration there is no increase in the thyroid uptake of ^{131}I in case of primary hypothyroidism, whereas the uptake greatly increases in cases of secondary hypothyroidism. The secondary hypothyroidism is reflected by an increase in blood concentrations of thyroxine (T_4), the primary hormone secreted by thyroid gland, after TSH administration. The dose and testing time vary, but generally T_4 concentrations are measured before and at 6 hours (4 to 8 hours) after TSH administration. In primary hypothyroidism, the post-TSH serum T_4 concentrations remain below the normal range for basal T_4 and rarely increase more than 0.2 µg/dl above the baseline. In secondary hypothyroidism, on other hand, the post-TSH serum T_4 concentrations either increase by at least 2 µg/dl over the basal serum T_4 value or exceed the normal range of T_4 concentration by 6 hours after TSH injection.

The TSH stimulation test is useful for dogs but has not proved successful in the diagnosis of hypothyroidism in cats. Repeated TSH stimulation test (daily for 3 consecutive days) is also used to restore the responsiveness of thyroid gland in animals in which the gland has become hypoactive due to lack of TSH. Because of limited availability of TSH and its high cost, the TSH stimulation test is not frequently used in veterinary medicine.

Dose

Bovine TSH

Dogs : 0.1 IU/kg up to a maximum dose of 5 IU (total), IV. For repeated stimulation test, the dose should be administered once daily for 3 consecutive days.

Recombinant human thyroid stimulating hormone

Recombinant human thyroid stimulating hormone (rhTSH) is biosynthetic TSH produced and is in clinical practice since 1998. Its mechanism of action and pharmacological properties are similar to those of endogenous TSH. The rhTSH has been approved mainly for the diagnostic monitoring of differentiated thyroid cancer patients. There are many other potential uses for rhTSH, including facilitation of treatment of patients with thyroid cancer and nodular goitre.

Protirelin

Protirelin is a synthetic tripeptide that is believed to be structurally identical to the naturally-occurring thyrotropin-releasing hormone produced by the hypothalamus. Chemically, it is 5-oxo-L-prolyl-L-histidyl-L-proline amide. Protirelin is used mainly for diagnostic purposes to differentiate pituitary and hypothalamic causes of hypothyroidism. As a valid TSH assay is not available for dogs and cats, interpretation of results is usually based on thyroid hormones. As with the TSH stimulation test, serum T_4

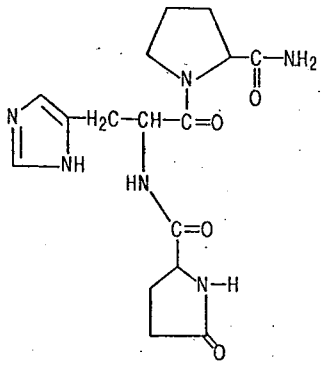


Fig. 49.6. Chemical structure of protirelin.

(not T_3) concentrations can be compared at baseline and 4 to 6 hours after administration of IV protirelin. Although the protocol for TRH stimulation varies and has not been definitively established, a mean increase of T_4 of 0.5 µg/dl above baseline has been reported in euthyroid (normal thyroid function gland) dogs. Failure to respond adequately as defined by the thyroid estimations, suggests hypothyroidism.

High doses of protirelin are associated with side effects like salivation, miosis, tachycardia and tachypnoea. Protirelin is clinically used in veterinary medicine for diagnosis of hypothyroidism in dogs and cats and also for diagnostic purposes in the evaluation of equine pituitary adenoma. It is also used as a diagnostic tool in hyperthyroidism in cats (in hyperthyroidism, there is a blunted response to protirelin because the raised blood T_4 concentration has a negative feedback on the anterior pituitary). Similar to TSH, high cost and non-availability of protirelin precludes its use in veterinary medicine.

Dose

Dogs & cats : 0.2 mg/kg, IV.

Horses : 1 mg (total), IV.

Note: Stimulation tests are usually done when hypothyroidism is suspected; these are less used for hyperthyroidism.

GONADOTROPIC HORMONES

The gonadotropic hormones (gonadotropins, gonadotrophins) are a family of protein hormones, which act on gonads and promote their growth and development. These hormones include anterior pituitary gonadotropins namely the follicle stimulating hormone and luteinising hormone, and non-pituitary gonadotropins namely the human chorionic gonadotropin (hCG) and equine chorionic gonadotropin (eCG).

Pituitary Gonadotropins

Follicle stimulating hormone and Luteinising hormone

The anterior pituitary secretes two gonadotropins viz. follicle stimulating hormone (FSH) and luteinising hormone (LH). In both male and female, FSH and LH are synthesised and secreted by gonadotroph cells in the anterior pituitary. The FSH stimulates growth of follicles in females and supports spermatogenesis in males. The LH is necessary for follicular maturation, oestrogen secretion and ovulation and luteinisation in females, and androgen production in males.

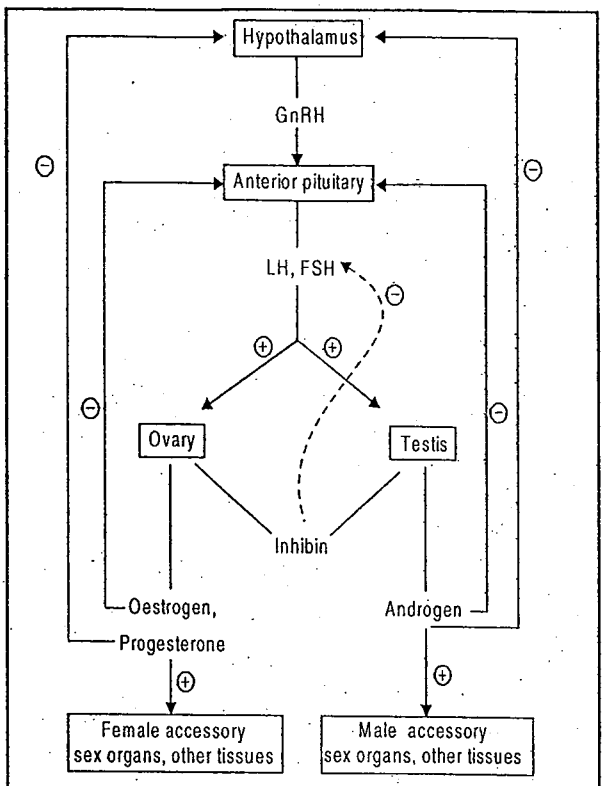


Fig. 49.7. Schematic representation of secretion, function and regulation of gonadotropins (See text for detail).

Abbreviations : GnRH = gonadotropin releasing hormone ; LH = luteinising hormone ; FSH = follicle stimulating hormone ; + = stimulation ; – = inhibition. Inhibin has inhibitory effect only on FSH secretion.

Chemistry

The pituitary gonadotropins FSH and LH are relatively large glycoproteins each containing two covalently bound structural subunits (α and β). The biological specificity depends on the β-subunit that is distinct, species specific, confers binding specificity and determines biological activity. The α-subunits are identical to each other and to other anterior pituitary glycoproteins (e.g. TSH) and are similar between mammalian species. Both α and β subunits are heavily glycosylated (23-28%) and are required for full biological activity. The deglycosylated glycoprotein hormones behave as antagonists (or partial agonists) of their glycosylated counterpart.

The FSH has a molecular weight of about 29,000 in pigs and 32,000 in sheep and has a half-life of 2-4 hours. The LH has molecular weight of 30,000 in sheep and cattle, contains 216 amino acids, and has a half-life of 1-3 hours. The pituitary content of LH is highest in cattle, sheep and cats, and lowest in horses. The overall size of these glycoproteins precludes economic synthetic production of these hormones.

Regulation of release

The synthesis and secretion of both FSH and LH by anterior pituitary is positively regulated by a single hypothalamic-releasing hormone called gonadotropins releasing hormone (GnRH), previously called FSH/LH-releasing hormone or simply LHRH. Although it is difficult to explain how hypothalamus activates a divergent pattern of FSH and LH secretion through a single releasing hormone, several factors like frequency and amplitude of GnRH pulses may determine whether FSH or LH, or both will

be secreted as well as the amount of each. The gonadotropins are negatively regulated by feedback effects of the gonadal steroids (progesterone and oestrogen in females and testosterone in males), which either produce inhibition of GnRH release from the hypothalamus and/or directly act on the pituitary (Figure 49.7). Large concentrations of oestrogen inhibit secretions of both gonadotropins, whereas low oestrogen concentration stimulates LH and FSH surges (the pre-ovulatory rise in oestrogen level, however, paradoxically stimulates LH and FSH secretion). Inhibin, a peptide produced in both ovaries and testes in response of FSH, selectively suppresses the synthesis and secretion of FSH with no known effect on LH. In contrast, dopamine inhibits only LH release. In some species, FSH secretion is also influenced by environmental conditions, changing season, day light length, etc.

The release of FSH and LH are pulsatile in response to pulses of GnRH in both female and male. In females, the secretion pattern of these hormones differs depending on the species and the phase of ovarian cycle. In bitches, LH is at basal levels during anoestrus, most of proestrus and dioestrus, but increases in both frequency and amplitude before proestrus (LH pulse) and before ovulation (preovulatory surge of duration 1 to 3 days). The FSH secretion in bitches surges to maximal level before ovulation in concert with previously LH surge, and remain relatively higher during dioestrus/pregnancy and also during anoestrus. In queens, LH release begins within minutes after copulation. In cattle, the FSH levels are pulsatile but surges are observed on days 17 and 18 before ovulation; ovulatory LH surge occurs on the day of oestrus. In mares, the gonadotropins surge is less acute and consists of gradual rise in LH during oestrus with maximum values being reached post-ovulation.

At birth, gonadotropins levels are undetectable (probably due to inhibition of gonadal steroids provided by placenta), increase shortly after birth and remain elevated for a few months, then decrease and remain suppressed until puberty. During puberty, GnRH secretion is initiated thus triggering the secretion of LH and FSH. Unlike the cycling females, the levels of FSH and LH remain practically constant (LH > FSH) and at a lower level in males.

Mechanism of action

The actions of FSH are mediated by the FSH receptors and those of LH by LH receptors (also called LH/CG receptors), which are present on the cell surface of their target cells. Both are G-protein coupled receptors which on activation increase cAMP production in the cell cytoplasm. The enhanced cAMP concentration causes formation of cAMP dependent protein kinase, which in turn causes phosphorylation of key enzymes involved in gametogenesis and conversion of cholesterol to pregnenolone synthesis. At very high concentration, the gonadotropin receptors also cause a stimulation of phospholipase C activity resulting in increase in breakdown of phospatidylinositol phosphates and increase intracellular Ca^{++} and protein kinase C activity. The actions mediated by high concentrations of gonadotropins are still unclear.

In ovaries, FSH receptors are present only on granulosa cells, while LH receptors are widely distributed on interstitial cells, theca cells, preovulatory granulosa cells and luteal cells. In testes, FSH receptor is expressed on seminiferous (Sertoli) cells, while LH receptor is expressed on interstitial (Leydig) cells. The LH receptors can also bind human chorionic gonadotropins (hCG). Thus at the cellular level, LH and hCG hormones are roughly equivalent.

Physiological effects

The primary physiological effect of the gonadotropins is the promotion of gametogenesis and/or gonadal steroid production. Thus, they control oestrous cycle in females and testicular function in males.

Ovaries: The ovarian changes during the oestrous cycle depend completely on the FSH and LH secreted by anterior pituitary. The overall actions of FSH and LH on ovaries are complex. In general, the FSH is responsible mainly for growth of follicles and synthesis of oestrogen, whereas the LH induces ovulation and stimulates progesterone production.

FSH: At the beginning of follicular phase of each cycle, FSH promotes growth and development of several follicles by binding to the granulosa cells. It also activates the aromatase system in granulosa cells, which converts androgens (mainly testosterone and androstenedione) to oestrogen (oestradiol-17 β). Androgens in turn are synthesised *de novo* in the theca cells in response to LH and are made available to granulosa cells via diffusion from adjacent theca cells.

LH: In females, the LH is responsible for final maturation and ovulation by binding to the thecal cells of the developing follicle. As the pre-ovulatory follicle(s) grow(s), oestrogen production increases, which through a positive feedback effect on LH and FSH release causes a preovulatory gonadotropic surge. The LH surge causes the rupture of preovulatory follicle(s) and release of ovum (Ova). Following ovulation, LH binds to granulosa cells and initiates the luteinisation process, so that the ovulated follicle forms the corpus luteum (CL), the transient endocrine gland. The LH binds to luteal cells and stimulates steroidogenesis resulting in increased progesterone (also oestrogen) production, which is essential for pregnancy. The process of luteinisation (change from oestrogen to progesterone secretion by granulosa cells) begins with the onset of the preovulatroy LH surge and accelerates with ovulation. In most domestic species, significant production of progesterone by the CL begins within 24 hours of ovulation. For most domestic species, LH is the important luteotropic hormone; in rodents, bitches, queens and sheep prolactin also acts as an important luteotropin.

During the luteal phase of the oestrous cycle, LH and FSH concentrations remain low due to the combined negative feedback effect of progesterone and also oestrogen on the hypothalamic-hypophyseal axis. The secretion of the luteolytic agent $PGF_{2\alpha}$ from the uterine endometrium in non-pregnant large domestic animals is responsible for regression of the CL and decreased progesterone secretion. This initiates enhanced secretion of gonadotropins and onset of next follicular growth and ovulation. In canines, spontaneous regression of CL and decline of progesterone marking the end of dioestrus result in increased level of prolactin, causing clinical signs of pseudopregnancy.

Testes: In males, LH stimulates the *de novo* synthesis of androgens, primarily testosterones, by interstitial cells (in synergy with FSH and probably prolactin) and is designated as the interstitial cell stimulating hormone (ICSH). The secreted testosterone is required for gametogenesis, maintenance of sexual libido and secondary sexual characteristics. The FSH, on the other hand, is not involved in gonadal steroid production, but is essential for normal sperm production. The FSH supports spermatogenesis and has a tropic influence on seminiferous tubules. Although the exact mechanism by which FSH promotes

spermatogenesis is not clear, it probably acts by stimulating Sertoli cells to synthesise proteins and nutrients needed by maturing sperms. Sertoli cells also produce an androgen binding protein, which serves as a carrier for testosterone. Testicular (also ovarian) atrophy occurs in absence of FSH.

Gonadotropins releasing hormone

Gonadotropins releasing hormone (GnRH) is a hypothalamic hormone that is responsible for regulating the synthesis of FSH and LH by the anterior pituitary. It is a decapeptide with the same amino acid sequence in all mammals.

After pulsatile release from the hypothalamus, GnRH traverses the hypothalamic-hypophyseal portal system and activates anterior pituitary gonadotroph receptors, which are members of the family of G-protein coupled receptors. The binding of GnRH to its receptors provokes the influx of extracellular Ca^{++} into cells and also stimulates the formation of inositol triphosphate that mobilises intracellular Ca^{++} and diacylglycerol. In response to increased intracellular Ca^{++} and diacylglycerol, both calmodulin and protein kinase C are activated, which in turn stimulate the release of preformed LH and FSH into circulation and also synthesis of new gonadotropins. After receptor activation, the GnRH is rapidly inactivated and cleared.

The GnRH is released from the hypothalamus in a pulsatile fashion and determines whether FSH and/or LH is secreted and the relative amount of each. The gonadal steroids can negatively modulate GnRH secretion (Figure 49.5). However, as discussed earlier, oestrogen can positively control the pre-ovulatory surge of LH and FSH in females. Although a pulsatile release of GnRH is required for the release and synthesis of LH and FSH, a continuous secretion or exogenous infusion of GnRH suppresses gonadotropin secretion by desensitising gonadotropins. Several other hormonal, neural, metabolic and environmental factors including norepinephrine, dopamine, serotonin, γ-aminobutyric acid, glutamic acid, acetylcholine (all central neurotransmitters), melatonin, and vasotocin (pineal gland secretions) have been reported to modulate GnRH release. Prolonged strenuous exercise or severe weight loss causes an inhibition of GnRH release.

GnRH and structurally related compounds have also been found in the uterus and ovary.

Non-Pituitary Gonadotropins

Gonadotropins which originate from sources other than pituitary are called non-pituitary gonadotropins or anterior-pituitary like gonadotropic hormones. These are secreted mainly by placenta and are responsible for the maintenance of corpus luteum of pregnancy in some species. The non-pituitary gonadotropins include human chorionic gonadotropin and equine chorionic gonadotropin.

Human chorionic gonadotropin

Human chorionic gonadotropin (hCG/HCG) or chorionic gonadotropin (CG) is a complex glycoprotein excreted in the urine of women during pregnancy. It has a molecular weight of about 40,000 and consists of both α and β subunits, which are present in the pituitary gonadotropins. Before implantation, it is secreted by the trophoblastic cells of blastocyst, but after implantation secretion of hCG comes from syncytiotrophoblasts of placental chorion. It can be detected in pregnant woman plasma and urine as early as 8 days after fertilisation of an ovum and maintained during gestation until delivery.

The hCG has a potent LH like effect. It binds to the LH receptors with equal avidity and maintains the corpus luteum of pregnancy and promotes secretion of oestrogen and progesterone by the corpus luteum. It also exerts an interstitial cell-stimulating effect on the foetal testes, thus resulting in production of testosterone in male foetus until the time of birth. The physiological action of hCG is indistinguishable from that of LH. It has half-life of 12-24 hours. Levels of hCG may be measured in the blood or urine mainly as a pregnancy test, intended to indicate the presence or absence of an implanted embryo. Testing for hCG may also be done when diagnosing or monitoring germ cell tumours and gestational trophoblastic disease.

Equine chorionic gonadotropin

Equine chorionic gonadotropin (previously called pregnant mare's serum gonadotropin, PMSG) is a complex glycoprotein hormone produced by the endometrial cups of the pregnant mare uterus. It has a molecular weight of about 62,000, possesses both α and β subunits and is very similar in composition to LH and FSH. It is present in pregnant mare from day 40 to about day 150 of pregnancy with peak concentration at about 80th day. It is responsible mainly for the maintenance of the corpus luteum of pregnancy in the mare.

The effects of eCG are similar to both luteinising hormone and, more predominantly, follicle stimulating hormone secreted by the anterior pituitary. Unlike pituitary gonadotropins, eCG has longer half-life of 2-5 days because it does not pass through the renal filter and remains in circulation for longer period.

DISORDERS OF GONADOTROPINS

Deficiency of Gonadotropins

Disturbances of gonadotropins secretion from pituitary may be responsible for delayed puberty in both males and females. In both male and female animals, a low or undetectable level of LH and FSH are indicative of hypogonadotropic hypogonadism and occurs due to hypothalamic disease or pituitary failure. In females, deficiency of FSH results in improper follicular growth and ovulation. Inadequate secretion of LH by pituitary may lead to ovulation failure or delayed ovulation, which often results in reduced conception rates and infertility in animals. In women, inadequate gonadotropins result in amenorrhoea and sterility. In males, deficiency of gonadotropins decreases libido and causes oligozoospermia and infertility.

Excess of Gonadotropins

Excess of gonadotropins may result in precocious puberty in both sexes. Ovarian hyperstimulation, superovulation and polycystic ovary may be observed in females. Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of male hormones (androgens), particularly testosterone, either through the release of excessive luteinizing hormone (LH) by the anterior pituitary gland or through high levels of insulin in the blood (hyperinsulinaemia).

TREATMENT OF GONADOTROPIN DISORDERS

Drugs are available for treatment of both gonadotropins deficiency and excess. These drugs are widely used in small and large animal practices for treatment of various reproductive disorders, cancers and/or synchronization of oestrus.

Classification

Drugs used to treat gonadotropins disorders have been broadly classified into two classes: drugs used in deficiency of gonadotropins and drugs used in excess of gonadotropins.

I. Drugs used in deficiency of gonadotropins
   1. Gonadotropins and analogues
      a. Pituitary gonadotropins
         e.g. follicle stimulating hormone-pituitary, luteinising hormone-pituitary, menotropin and urofollitropin.
      b. Non-pituitary gonadotropins
         e.g. human chorionic gonadotropin and equine chorionic gonadotropin.
   2. Gonadotropin releasing hormone and analogues
      e.g. gonadorelin.
   3. Gonadotropin releasing hormone-receptor agonists
      e.g. buserelin, leuproline, goserelin deslorelin, histrelin, nafarelin and triptorelin.

II. Drugs used in excess of gonadotropins
   1. Gonadotropin releasing hormone receptor antagonists
      e.g. ganirelix, degarelix, abarelix and cetrorelix.
   2. Synthetic gonadal steroids
      e.g. danazol and gestrinone.

I. Drugs Used In Gonadotropins Deficiency

1. Gonadotropins and Analogues

Gonadotropins are commercially available as pituitary gonadotropins and non-pituitary chorionic gonadotropins. In veterinary medicine, chorionic gonadotropins are used more frequently than cadaver pituitary gonadotropins because of their easier availability and lower cost. Preparations of LH and FSH derived from pituitaries are no longer used in human medicine because of possible contamination with a virus and due to shortage of material.

1. Pituitary Gonadotropins

Follicle stimulating hormone-Pituitary

Follicle stimulating hormone-Pituitary (FSH-P) is available commercially for use in veterinary species. It is obtained from the pituitary gland of food producing animals. Additionally, purified human FSH is also available. Porcine or ovine follicle stimulating hormone is widely used in livestock industry for superovulation in cattle. It is commercially available as lyophilised powder, which needs reconstitution before use.

Chemistry

Follicle-stimulating hormone-Pituitary is obtained from cadaver pituitary hence it contains essentially the same chemical structure as synthesised in body. The FSH-P may also have small amounts of luteinising hormone present. One mg of FSH-P has been standardised to 1 Armour Unit. One Armour Unit can contain 9.4-14.2 International Units (IU) of FSH.

Pharmacological effects

The mechanism of action and pharmacological effects of FSH-P are similar to those of endogenous FSH produced by anterior pituitary gland. It stimulates multiple follicle growth in ovaries and oestrogen production in the female. In males, the action is mainly seen on seminiferous tubules leading to spermatogenesis, if androgen production by Leydig cells has occurred.

Pharmacokinetics

The pharmacokinetic data on FSH-P are not sufficiently available in domestic animals. It has a short half-life of 2 to 4 hours. It is generally given subcutaneously and depending on dose may elevate plasma FSH concentration.

Side effects/Adverse effects

Follicular stimulating hormone-P causes cystic endometrial hyperplasia, undesirable superovulation and follicular cysts. These adverse effects are potentially high when FSH-P is used in high dosages or repeatedly. Repeated dose of porcine FSH-P in cattle may lead to immune-mediated reduced effects and occasional anaphylactic reactions.

Contraindications and precautions

The FSH-P should not be used in animals with pre-existing endometrial hyperplasia or follicular cysts. It should be used only when required; inadvertent use of FSH-P should be avoided.

Clinical uses

Follicular stimulating hormone-P is used in veterinary medicine mainly to induce superovulation in cattle and for the purpose of out-of-season breeding in other species. In males, it may improve libido and spermatozoa count and can be used to treat infertility. It can also be used in various species as a supplemental source of FSH, when there is a general deficiency of the hormone. Presently, the clinical use of FSH-P in veterinary practice has declined because it is not freely available and is also expensive.

Dose

For induction of superovulation

Cattle : 25 IU (total), IM, 2 times daily for 3 days in the luteal phase (10-14 cycle days). Animal is given $PGF_{2\alpha}$ at the time of 3rd injection to induce oestrus and breeding/AI done on 4-22 hours after onset of oestrus. Embryos are recovered from donor cows 6-8 days after breeding/artificial insemination.

For induction of Oestrus

Dogs : 20 IU/kg, SC, daily for 10 days. Pro-oestrus usually occurs between days 7-10 and lasts 2-3 days. Animal is given hCG (500 IU, SC) on days 10 and 11 and breeding done on days 12, 14, and 16.

Cats : 2 mg, IM, daily for up to 5 days until oestrus is observed. This is followed by hCG on 1st and 2nd day of oestrus.

For indications of FSH deficiency

Dogs : 5-15 mg (total), IV, IM or SC.

Cattle & horses: 10 – 50 mg (total), IV, IM or SC.

Sheep & pigs : 5 – 25 mg (total), IV, IM or SC.

Luteinising hormone-Pituitary (LH-P)

Luteinising hormone-P is available from domestic animal pituitary gland but is less frequently used in veterinary medicine. It can be used in female animals to control ovulation and to treat persistent infertility, whereas in male animals it is occasionally used to treat cryptorchidism. In males it increases testosterone production that causes the descent of testicles into the scrotum. It may or may not be commercially available due to its limited production.

Menotropin

Menotropin (also called human menopausal gonadotropin or HMG) is obtained from the urine of post-menopausal women and contains both follicle stimulating hormone and luteinising hormone. Often, it contains human chorionic gonadotropin (hCG) as well. This drug was originally designed for use in women where it stimulates ovaries to produce multiple follicles, thus making them more fertile. It is occasionally used in veterinary medicine, mainly for induction of superovulation. It has both follicle stimulating and luteinising activities.

Urofollitropin

Urofollitropin (uFSH) is a menotropin preparation from which most of the LH and other proteins has been removed. Therefore, it is primarily a purified form of follicle-stimulating hormone. In human medicine, urofollitropin is preferred over the combined FSH and LH preparation for induction of ovulation in women with polycystic ovarian disease because such patients have elevated LH/FSH ratio. It is administered by subcutaneous injection, often in combination with human chorionic gonadotropin (hCG) to assist in ovulation and fertility. It is also used with *in vitro* fertilization methods. Important side effects include mild diarrhoea, nausea or vomiting, shortness of breath and constipation.

2. Non-Pituitary Gonadotropins

Non-pituitary gonadotropins or chorionic gonadotropins are widely used in both human and veterinary medicines and have virtually replaced the pituitary gonadotropins. Non-pituitary gonadotropins are cheap, easily available and effective enough to be used clinically in various species.

Human chorionic gonadotropin

Human chorionic gonadotropin (hCG, HCG, chorionic gonadotropin, pregnancy-urine hormone) is a glycoprotein hormone secreted by the placenta and obtained from the urine of pregnant women. It is commercially available as a white amorphous, lyophilised powder, which is soluble in water and insoluble in alcohol. One mg of hCG contains 1500 IU. The hormone is chemically different from pituitary LH but its activity is primarily LH-like, with some effects similar to those caused by FSH.

Pharmacological effects

Human chorionic gonadotropin has similar effects to luteinising hormone secreted by the anterior pituitary in both male and female animals. It occupies the LH/CG receptors present in gonads and mimics quite closely the effects of LH/ICSH. In female animals, hCG stimulates corpus luteum to produce progesterone and can induce ovulation possibly also in patients with cystic ovaries. In bitches, hCG induces oestrogen secretion. In males, hCG can stimulates the secretion of testosterone by interstitial testicular cells. Therefore, it increases libido and may also stimulate testicular descent when there is no anatomical obstruction.

Pharmacokinetics

Human chorionic gonadotropin is a polypeptide hence it is rapidly destroyed in GI tract after oral administration. After IM administration, it is distributed mainly to the ovaries in females and to the testes in males. Peak plasma levels are achieved at about 6 hours. It has a half-life of 12-24 hours, although duration of half-life varies with species. Due to long half-life, hCG is effective for 2-3 days in many species.

Side effects/Adverse effects

Important adverse effects associated with hCG usage in animals include hypersensitivity reactions and pain at the injection site. Occasional anaphylactic reactions may occur. It may induce abortion in mares prior to the 35th day of pregnancy, perhaps due to increased oestrogen levels.

Contraindications and precautions

Human chorionic gonadotropin is contraindicated in patients having a previous history of hypersensitivity reaction to hCG. Immune-mediated reduced effect after repeated doses is observed in animals. The hCG should not be used in pregnant animals.

Drug interactions

Human chorionic gonadotropin does not show significant interaction with commonly used therapeutic agents.

Clinical uses

In veterinary medicine, hCG is used to supplement or replace luteinising hormone in cases of ovulation failure and to delay or help predict timing of ovulation. In mares, hCG is used to induce ovulation in animals with prolonged oestrus and before mating or artificial insemination. In cattle, it is used for the treatment of nymphomania due to cystic ovaries. In male animals, hCG is used to improve libido with variable results and to help descent of testes in cryptorchids. Cryptorchidism of any species often responds to hCG therapy, provided the inguinal canal is patent and therapy commences early enough.

Human chorionic gonadotropin is also used as a diagnostic tool to identify various sexual disorders in both males and females. In males, hCG challenge test is used to identify the presence of retained testis in cryptorchids or to determine if testicular tissue remains in castrated animal. A rise of serum testosterone level in response to hCG confirms presence of testicular tissue in both conditions. In females, it is used to diagnose sexual differentiation disorders or to determine if functional ovarian tissue remains after ovariohysterectomy. In females with suspected sexual differentiation disorder, testosterone level is taken as an indicator, whereas elevation of progesterone level indicates functional ovarian tissue.

Dose

For therapeutic purposes

For induction of ovulation/Ovulation failure

Bitches	:	100 – 500 IU, IM on day of mating.
Queens	:	250 IU, IM on days 1 and 2 of oestrus.
	:	50 – 75 IU, IM immediately after insemination; drug and insemination is repeated in 24 hours.
Mares	:	1500 – 3000 IU, IM or IV, 24 hours after mating or artificial insemination.

For luteinisation of a persistent follicular cyst/cystic ovarian disease/prolonged oestrus

Bitches	:	500 IU, IM. Drug is repeated in 24 hours. If effective, it will convert from prooestrus to oestrus in 1-2 days and sexual behaviour stops within 2 weeks.
Cattle	:	3000 IU, IV.
Mares	:	1000 – 5000 IU.
Sheep & goats	:	250 – 1000 IU, IV, or IM.

For cryptorchidism

Dogs : 500 IU, SC or IM, 2 times weekly for 4 to 6 weeks.

Foals : 1000 IU, IV or IM, 2 times weekly for 4 to 6 weeks.

For increasing libido

Dogs : 100 – 500 IU, IM, 2 times weekly for up to 6 week or 6 – 12 hours before mating (temporary effect).

For diagnostic purposes

To identify presence of retain testis in cryptorchids

Foals : 6000 IU, IV. Blood sample should be collected before and at 30 to 120 minutes after injection for testosterone level.

To determine if testicular tissue remains in castrated animal.

Dogs : 0.04 mg/kg, IM. Blood samples should be collected before and at 4 hours for testosterone level.

Cats : 0.25 mg (total), IM. Blood samples should be collected before and at 4 hours for testosterone level.

To determine if functional ovarian tissue remains after ovariohysterectomy

Bitches : 100 – 1000 IU, IM during apparent oestrus episode. Blood samples should be collected for progesterone level before and 5-7 days after treatment.

Equine chorionic gonadotropin

Equine chorionic gonadotropin (Serum gonadotropin, pregnant mare's serum gonadotropin, eCG, PMSG) is complex glycoprotein that is secreted by the endometrial cups of pregnant mare's uterus. It is extracted from the serum of mare during the first trimester of pregnancy. Its activity is primarily like anterior pituitary FSH, but it also has some actions similar to pituitary LH. It is commercially available as lyophilised powder for reconstitution with sterile water, normal saline or appropriate buffer.

Pharmacological effects

Equine chorionic gonadotropin, like all glycoprotein hormones, is composed of two dissimilar subunits named alpha and beta. The alpha subunit is common to all glycoprotein hormones (LH, FSH, TSH, CG), but the beta subunit is specific and is responsible for receptor binding specificity. The pharmacological effects of eCG in animals are similar to both luteinising hormone and, more predominantly, follicle stimulating hormone. It has been suggested that eCG produces LH like activity only in mares; in other species it has mainly FSH like activity. Thus in mares, eCG only luteinises follicles already present on the ovary but is not effective in stimulating follicular growth. On the other hand, it mainly stimulates follicular growth in other species.

Pharmacokinetics

Pharmacokinetics data on eCG in domestic animals are scarce. Being a glycoprotein, eCG is not active when administered orally. It has a long half-life of 2-5 days in different species. It has long period of action mainly because it does not pass through renal filter and remains circulating in body for long period.

Side effects/Adverse effects

In heterogeneous species, the most important adverse effect of eCG is occasional hypersensitivity or anaphylactic reactions. Immune mediated reduced effects are seen after repeated doses.

Clinical uses

Equine chorionic gonadotropin is used in veterinary medicine to induce follicular growth and ovulation either alone or following progesterone pre-treatment (requires progesterone priming). It is employed in combination with hCG to induce oestrus in bitches in anoestrus. It is also employed to induce superovulation in cattle used as donors in embryo transfer and in conjunction with a progesterone-releasing intravaginal device to stimulate cyclical activity in acyclical cows. It should be used carefully to superovulate cattle because it has long half-life which may stimulate non-ovulated or new follicles to produce high quantities of oestrogens early in the new cycle. These oestrogens increase the number of abnormal or degenerating embryos and reduce the number of transferable embryos obtained per donor. This can be overcome by use of anti-eCG antibodies to neutralise the circulating eCG or by the use of FSH-P. Equine chorionic gonadotropin can be used in males to increase libido and spermatogenesis.

Dose

For treatment of anoestrus

Dogs : 20 IU/kg, SC, daily for 10 days followed by hCG (500 IU, IM or IV) on day 10.

Pigs : 1000 – 15000 IU, SC or IM.

Cattle : 300 – 800 IU, SC or IM.

For induction of oestrus outside normal breeding season/For synchronise mating

Sheep : 300 – 800 IU, IM or SC.

Goats : 400 – 600 IU, IM or SC.

For induction of oestrus in acyclic animals

Cattle : 400 – 700 IU, SC or IM following treatment with progesterone.

For superovulation

Cattle : 1500 – 4000 IU, SC or IM, as per embryo transfer protocol.

2. Gonadotropin-Releasing Hormone and Analogues

Gonadorelin

Gonadotropin-releasing hormone (GnRH) is a hormone produced by the hypothalamus, which controls the secretion of FSH and LH by anterior pituitary. Commercially, GnRH is obtained from natural sources or is produced synthetically. Synthetic GnRH or drug form of GnRH is often referred to as gonadorelin.

Chemistry

Gonadorelin is a synthetic decapeptide prepared using solid phase peptide synthesis. It essentially contains the same sequence of amino acids as occurring naturally. It occurs as white or faintly yellowish-white powder and is available as the HCl or diacetate decahydrate salt. Fifty micrograms of gonadorelin acetate is approximately equivalent to 31 units. It is mostly marketed as lyophilised powder.

Pharmacological effects

Gonadorelin binds to the GnRH receptors on gonadotroph cells of anterior pituitary and causes release of both FSH and LH. The effects of gonadorelin on gonadotropin release are dependent on the

dose, route and frequency of injection and also on hormonal status of animal at the time of treatment. Generally a single injection of gonadorelin causes a surge-like release of FSH and LH, whereas a constant infusion causes a gradual LH and FSH release that returns to baseline after some time (a continuous infusion of GnRH suppresses gonadotropin release probably by desensitising gonadotrophs). Following gonadorelin injection, the FSH and LH release are higher when animal is under the influence of oestrogen, and lower when animal is under the influence of progesterone. This mainly occurs due to the fact that oestrogen can potentiate the release of gonadotropins during the follicular phase of oestrus cycle.

Pharmacokinetics

Like other peptide hormones, gonadorelin is ineffective after oral administration. After IV injection, gonadorelin is rapidly distributed to extracellular fluid. It has a very short half-life (7-12 minutes) as it is rapidly metabolised by anterior pituitary peptidases.

Side effects/Adverse effects

No significant side/adverse effects have been reported with gonadorelin. Gonadorelin is unlikely to cause hypersensitivity reactions in animals. No significant contraindication to gonadorelin has been indicated.

Clinical uses

In veterinary medicine, gonadorelin is indicated for the treatment of ovarian follicular cysts in cattle and to induce ovulation in horses, cattle and some other species. In mares, ovulation is induced in animals with prolonged oestrus during the transitional phase from winter anoestrus to the onset of normal cyclical ovarian activity and also after mating. Gonadorelin has been used in cattle to reduce the time interval from calving to first ovulation and to overcome delayed ovulation, particularly where this syndrome is suspected of causing reduced conception rates in high yielding post-partum cows bred within 3 months of parturition. In cats, it is used as an alternate therapy to FSH or hCG to induce oestrus in queens with prolonged anoestrus.

Gonadorelin is used also as a diagnostic tool to identify reproductive disorders in both man and animals. In dogs, it is used to identify intact animal versus castrated animal by stimulating FSH and LH production and then testicular steroidogenesis and to test pituitary insufficiency. In human medicine, gonadorelin has been used for the diagnosis of hypothalamic-pituitary dysfunction, cryptorchidism and depression secondary to prolonged severe stress.

Dose

For treatment of ovarian cysts

Cattle : 0.1 – 0.5 mg (total), IM. Dose may be repeated, if required.

To induce ovulation

Cats : 0.025 mg (total), IM after mating.

Sheep & goats : 0.1 mg (total), IM, daily for 4 to 5 days for induction of ovulation out of breeding season.

Cattle : 0.25 mg (total), IM, in conjunction with artificial insemination.

Anoestrus post-partum

Cattle : 0.5 mg (total), after 40 days post-partum. It may be repeated after 1-3 weeks.

To help descent of cryptorchid testes

Dogs : 0.05 – 0.1 mg (total), SC or IM. Dose may be repeated after 4 to 6 days, if no improvement is observed.

GnRH challenge test to diagnose pituitary insufficiency or testicular steroidogenesis.

Dogs : 125 - 250 ng (nanogram)/kg, IV.

3. Gonadotropin Releasing Hormone-Receptor Agonists

A number of GnRH receptor agonists (also called GnRH agonists) has been synthesised recently by chemical modification of the GnRH molecule. These agents usually have greater biological activity and longer half-life than natural or synthetic GnRH because of higher binding for the receptor and resistance to the enzymatic hydrolysis. These agents initially increase gonadotropins release from the anterior pituitary, but after continuous use (1-2 weeks) they inhibit FSH and LH secretion and suppress gonadal function because their continued presence in body causes desensitisation and down regulation of GnRH receptors. Recovery of testicular or ovarian steroidogenesis occurs within 2 months of stopping GnRH analogue treatment.

GnRH-receptor agonists are frequently used in human medicine but have limited application in animals because they are expensive and not freely available.

Buserelin

Buserelin is a synthetic analogue of GnRH. Buserelin acts as an agonist at pituitary GnRH receptors. It is about 20 times more potent than GnRH and is comparatively longer acting than endogenous GnRH. It initially increases the gonadotropins release from anterior pituitary by causing activation of GnRH receptor, but after chronic and continuous administration, usually 2 to 4 weeks after initiation of therapy, a sustained decrease in LH and FSH secretion and marked reduction of testicular and ovarian steroidogenesis is observed.

Buserelin is used mainly in mares for induction of ovulation or stimulation of cystic oestrus and in cattle for treatment of follicular cyst and improvement in conception rate. In human beings, is used in the treatment of hormone-responsive cancers such as prostate cancer and breast cancer, oestrogen-dependent conditions such as endometriosis or uterine fibroids, and in assisted reproduction. It is also used in fish farming industry. Overall, its use in veterinary medicine is less.

Dose

For induction of ovulation

Cattle : 0.01 mg (total), SC or IM (preferred), 6 to 8 hours before or at time of insemination.

Horses : 0.04 mg (total), SC, IM (preferred), or IV, 6 hours before insemination. Dose may be repeated after 1 day, if required.

For treatment of follicular cysts/Anoestrus

Cattle : 0.02 mg (total), SC, IM (preferred), or IV. Dose may be repeated after 8-22 days (anoestrus) or 10-14 days (follicular cysts), if required.

Leuproline

Leuproline (Leuprolide) is a synthetic GnRH receptor agonist. Like buserelin, it initially stimulates the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from pituitary, but on long-term use down regulates the secretion of both FSH

and LH leading to a dramatic reduction in sex hormonal levels. Leuproline has been approved mainly for use in human patients for treatment of precocious puberty, hormone-responsive cancers such as prostate cancer and breast cancer, oestrogen-dependent conditions such as endometriosis or uterine fibroids and to control ovarian stimulation *In Vitro* Fertilization (IVF).

Goserelin

Goserelin acetate is another GnRH receptor agonist. In comparison to buserelin and leuproline, goserelin has more affinity for LHRH receptors and hence has more specific action on the release of LH; therefore is sometimes also called luteinizing hormone releasing hormone (LHRH) agonist. By binding to LHRH receptors in the pituitary gland, goserelin causes an initial increase in production of luteinizing hormone and thus leading to an initial increase in the production of corresponding sex hormones. However, the production and release of sex hormones testosterone and oestrogen occurs in a non-pulsatile (non-physiological) manner. On long-term use, the constant exposure to goserelin causes down regulation of LHRH receptors with decreased production LH. This causes the disruption of the endogenous hormonal feedback systems, resulting in the down-regulation of testosterone and oestrogen production. Eventually, after a period of about 14–21 days, production of sex hormones is generally reduced to castrate levels. Similar to other GnRH agonists, goserelin acetate is used mainly in human medicine to treat hormone-sensitive cancers of the prostate and breast, precocious puberty and some benign gynaecological disorders (endometriosis, uterine fibroids and endometrial thinning).

Other GnRH-receptor agonists

Other GnRH receptor agonists used in human medicine (some also in veterinary practice) include deslorelin, histrelin, nafarelin and triptorelin (Table 49.2). Goserelin acetate is designed for subcutaneous injection with continuous release over a 28 day period, whereas nafarelin acetate is administered as a nasal spray. Depot preparation of leuproline has been produced that is given once in a month. Long-acting GnRH analogues are mainly used for the treatment of a variety of endocrine disorders in human medicine.

II. Drugs Used In Excess of Gonadotropins

1. Gonadotropin Releasing Hormone Receptor Antagonists

Gonadotropin releasing hormone-receptor (GnRHR) antagonists are drugs which inhibit action of GnRH on its receptors. These agents are synthesised by chemical modification of the GnRH molecule. Similar to endogenous GnRH, they bind to GnRH receptors on gonadotroph cells in the anterior pituitary, however they do not result in stimulation of gonadotropins secretions. Unlike the GnRH receptor agonists, they decrease gonadotropins (LH and FSH) release from the anterior pituitary without causing their initial elevation. The early GnRH antagonists had the limitation producing reactions due to histamine release, but more recent agents (e.g. ganirelix) have low histamine releasing potential with full GnRH antagonistic potency.

Ganirelix

Ganirelix acetate (or diacetate) is a synthetic decapeptide with gonadotropin-releasing hormone receptor (GnRHR) antagonist properties. It is derived from the native GnRH with substitutions of amino acids at positions 1, 2, 3, 6, 8 and 10. Ganirelix competes with natural GnRH for binding to GnRH receptors in the pituitary gland. This reversible blinding blocks the release of LH and FSH from the pituitary. The reduction in LH subsequently leads to a rapid and sustained suppression of sex hormones from the gonads. Ganirelix acetate has been approved mainly for use in human medicine for decreasing the premature LH surges in women undergoing controlled ovarian hyperstimulation. Ganirelix has been reported to be safe, but rare cases of hypersensitivity reactions, including anaphylactoid reactions with the first dose, have been noted. Ganirelix acetate injection is supplied as a colourless, sterile, ready-to-use, aqueous solution intended for subcutaneous administration only.

Degarelix

Degarelix is another synthetic peptide obtained from the chemical modification of the natural GnRH. Similar to ganirelix, it reversibly binds with the gonadotropin-releasing hormone receptor in the pituitary gland and blocks the action of endogenous GnRH. This induces a fast and profound reduction in levels of gonadotropins LH and FSH, which in turn reduce levels of gonadal sex hormones, in particular the testosterone release from testes. Degarelix has been approved mainly for the treatment of prostate cancer in human beings. Degarelix produces a rapid onset of action and leads to a rapid and sustained suppression of testosterone release from the testes with subsequent fast reduction in size and growth of the prostate cancer.

Other drugs

Some other GnRH receptor antagonists available for clinical use in human medicine are abarelix and cetrorelix. These drugs are recommended for treatment of hormone-sensitive cancers of the prostate and/or breast, and some benign gynaecological disorders (endometriosis, uterine fibroids and endometrial thinning).

Table 49.2: Chemical structures and relative potency of GnRH and some GnRH-receptor agonists.

Drug	Relative Potency	Amino Acid Sequence
		1 2 3 4 5 6 7 8 9 10
GnRH	1	Pyro Glu – His – Trp – Ser – Tyr – Gly – Leu – Arg – Pro – Gly-NH_2
Leuproline	15	Pyro Glu – His – Trp – Ser – Tyr – D-Leu – Leu – Arg – Pro – N-EtNH_2
Buserelin	20	Pyro Glu – His – Trp – Ser – Tyr – D-Ser(tBu) – Leu – Arg – Pro – N-EtNH_2
Deslorelin	144	Pyro Glu – His – Trp – Ser – Tyr – D-Trp – Leu – Arg – Pro – N-EtNH_2
Nafarelin	200	Pyro Glu – His – Trp – Ser – Tyr – D-Nal(2) – Leu – Arg – Pro – N-EtNH_2
Histrelin	210	Pyro Glu – His – Trp – Ser – Tyr – D-His(ImB_2l)– Leu – Arg – Pro – N-EtNH_2
Triptorelin		Pyro Glu – His – Trp – Ser – Tyr – D-Trp – Leu – Arg – Pro-Gly-NH_2

Abbreviations: N-EtNH_2 = N-ethylamide; tBu = t-butyl; D-Nal(2) = 6[3-(2-naphthalenyl)-D-alanine]; ImB_2l = imidobenzyl.

2. Synthetic Gonadal Steroids

Gestrinone

Gestrinone is a synthetic steroid that acts as an anti-progestogen with some androgenic activity. Its mechanism of action consists of suppression of the release of gonadotropins from the anterior pituitary. It has also shown to interact with the oestrogen receptor, androgen receptor and progesterone receptor (see for detail Chapter 52). It is largely used for treatment of endometriosis in human medicine.

Danazol

Danazol (17alpha-ethinyl testosterone) is a derivative of the synthetic steroid ethisterone and a modified testosterone. Its action resembles gestrinone and is occasionally used for treatment of endometriosis. The detailed pharmacology of danazol has been given in Chapter 51.

ADRENOCORTICOTROPIC HORMONE

Adrenocorticotropic Hormone

Adrenocorticotropic hormone (Adrenocorticotrophic hormone, adrenocorticotropin, adrenocorticotrophin, corticotropin, corticotrophin, ACTH) is a polypeptide hormone secreted by the corticotrophic cells of the anterior pituitary. It regulates mainly the synthesis and secretions of glucocorticoids from the adrenal cortex. One mg of ACTH of international standard is equivalent to one USP unit of ACTH.

Chemistry

Adrenocorticotropic hormone is a single chain polypeptide hormone, which in the sheep, pig, cow and human contains 39 amino acids with molecular weight about 4500. The biological activity of ACTH resides in the first 24 amino acids, sequence of which is identical in all species studied so far. It is derived from a larger peptide 'pro-opio-melanocortin' (Mol. wt. 30,000), which also gives rise to endorphins, two lipotropins (LPH) and two melanocyte stimulating hormones (MSH) (see Chapter 17). The first 13 amino acids of ACTH are similar to those of MSH, which accounts for some overlapping activities.

Regulation of Secretion

The physiological release of ACTH from anterior pituitary is intimately associated with the hypothalamic corticotropin releasing hormone (CRH) and a feedback control mechanism. The ACTH release is stimulated by CRH whose release from hypothalamus is under neural control, particularly from the limbic system. A variety of stressful stimuli like cold, infections, severe pain, injury, toxins, anxiety, fear and blood loss cause release of CRH. Catecholamines and vasopressin also potentiate the action of CRH. On the other hand, secretion of ACTH is inhibited by glucocorticoids which exert an inhibitory feedback influence on the hormone by acting either directly on anterior pituitary or indirectly on hypothalamus (Figure 49.8). In pituitary, glucocorticoids act through the glucocorticoid receptors to inhibit the expression of pro-opio-melanocortin in corticotrophs, as well as the release of ACTH. These effects are both rapid (occurring within seconds to minutes) and delayed (requiring hours and involving changes in gene transcription). The ACTH concentration in blood also influences the CRH release through an inhibitory feedback mechanism, but it is less important. Dopamine appears to be important to the negative control of ACTH release from pituitary gland in dogs and some other species.

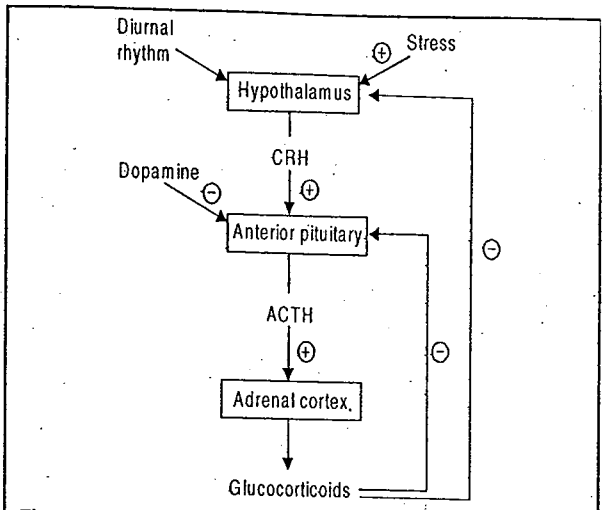


Fig. 49.8. Schematic representation of secretion, regulation and functions of adrenocorticotropic hormone (See text for detail).

Abbreviation : CRF = corticotropin releasing hormone ; ACTH = adrenocorticotropic hormone ; + = stimulation ; − = inhibition.

Secretion of ACTH from pituitary has a circadian rhythm in some species. The diurnal rhythm is influenced by higher neuronal centres in response to sleep-wake cycle, which affects mainly the negative feedback control system produced by glucocorticoids (glucocorticoids levels do not remain uniform in blood throughout the day). These changes result in rise in the secretory rates of CRH, ACTH and glucocorticoids concentration in morning and fall in evening in humans, pigs and horses, but reverse in nocturnal species. Dogs and cats do not show morning rise in glucocorticoids. Further, feedback inhibition also appears to be overpowered during stress because rise in ACTH secretion continues despite high plasma level of hydrocortisone induced by it.

Mechanism of action

Adrenocorticotropic hormone stimulates the ACTH specific G-protein receptors present in the middle and inner zones of adrenal cortex to synthesise glucocorticoids, primarily hydrocortisone and corticosterone. It causes a rise in intracellular cAMP concentration mediated through activation of adenylate cyclase enzyme in the cell membranes, which in turn activates the intracellular enzymes which cause formation of adrenocortical hormones. Activation of the enzyme protein kinase A, which causes initial conversion of cholesterol to pregnenolone (see for detail, Chapter 48) is the most important and rate limiting step of all the ACTH-stimulated steps for controlling adrenocortical secretion.

The response of adrenocortical cells in the middle and inner zones of adrenal cortex to the ACTH has two phases, an acute phase and a chronic phase. The acute phase occurs within seconds to minutes and reflects mainly an increased supply of cholesterol substrate to the steroidogenic enzymes for steroidogenesis. The chronic phase occurs over hours to days resulting largely from increased transcription of the steroidogenic enzymes with associated increase in the steroidogenic capacity of the gland. The long-term stimulation of the adrenal cortex by ACTH not only increases secretory activity, but also causes hypertrophy and proliferation of the adrenocortical cells.

Physiological effects

The primary physiological effect of ACTH is to stimulate the synthesis and secretion of glucocorticoids, mainly hydrocortisone and corticosterone, by the middle and inner zones of adrenal cortex. In addition small amounts of sex hormones are secreted, especially androgenic hormones, which exhibit about the same effects in body as the male sex hormone testosterone. Mineralocorticoids production by the outer zone of adrenal cortex is less influenced by pituitary ACTH. Adrenocorticotropic hormone is required for stimulation of synthesis, but not secretion of mineralocorticoids.

Adrenocorticotropic hormone, in addition to adrenal steroidogenesis, has a variety of metabolic effects like lipolysis, ketosis and hypoglycaemia, which are produced by high concentrations of hormone. ACTH also improves learning in experimental animals (an extra endocrine effect) mediated via distinct receptors in the CNS. High concentration of ACTH may cause hyperpigmentation probably by activation of MSH receptors on the melanocyte.

Adrenocorticotropic Hormone-Regulating Hormone

Corticotropin releasing hormone

Corticotropin releasing hormone (CRH), also called corticotropin releasing factor (CRF), is a peptide composed of 41 amino acids in sheep and man. The cell bodies of neurons which secrete CRH are located mainly in the paraventricular nucleus of the hypothalamus. It is secreted into the primary capillary plexus of hypophyseal portal system in the median eminence of hypothalamus and then carried to the anterior pituitary gland where it acts on CRH receptors on corticotroph cells to induce ACTH secretion. The CRH receptors exist in two isoforms viz. CRH-1 and CRH-2 receptors, each encoded by a separate gene. The CRH receptor on the corticotroph cells is a G-Protein coupled receptor, which increases ACTH synthesis as well as release through increased cAMP. The anterior pituitary gland can secrete only minute quantities of ACTH in the absence of CRH. In addition to being produced in the hypothalamus, CRH is synthesized in peripheral tissues, such as T lymphocytes and is highly expressed in the placenta.

DISORDERS OF ACTH

Deficiency of ACTH

Deficiency of ACTH resulting from pituitary insufficiency leads to hypoadrenocorticism (secondary hypocorticism). However, the occurrence of isolated ACTH deficiency in animals is rare; it occurs along with deficiency of other pituitary derived hormones. In patients suffering from pituitary dependent hypoadrenocorticism, the mineralocorticoid activity is generally maintained but glucocorticoids activity is severely depressed. Iatrogenic suppression of ACTH secretion and pituitary-adrenal axis is a more common form of abnormally encountered condition in animals due to indiscriminate and prolonged use of exogenous glucocorticoids in non-endocrine diseases (see for detail Chapter 51).

Excess of ACTH

Excess secretion of ACTH results mainly from pituitary tumours derived from corticotroph cells either in pars digitalis or in pars intermedia. It may also occur by the ectopic secretion of ACTH by a tumour elsewhere in the body such as an abdominal carcinoma. The excess secretion of ACTH results in hyperplasia of adrenal cortex cells and a clinical syndrome of hydrocortisone excess (hyperadrenocorticism or Cushing's disease). Hyperadrenocorticism is one of the most common endocrine diseases of the dogs and is mainly pituitary dependent. It occurs occasionally in horses, but is rare in other domestic species.

Depression of hydrocortisone secretion caused by primary atrophy of adrenal cortices (primary hypoadrenocorticism) also causes excess secretion of ACTH (also MSH) due to suppression of normal negative feedback to the hypothalamus. In human beings, the excess amount of ACTH in adrenal cortical insufficiency or Addison's disease cause hyperpigmentation effect because ACTH can stimulate formation of melanin by the melanocyte in the same way as the MSH does.

TREATMENT OF ACTH DISORDERS

A pharmacological approach to adrenocorticotropic hormone is an important part of modern medicine. Drugs are available for treatment of both ACTH deficiency and ACTH excess.

Classification

Drugs used to treat ACTH disorders have been broadly classified into two classes: drugs used in deficiency of ACTH and drugs used in excess of ACTH.

I. Drugs used in deficiency of ACTH
   1. Adrenocorticotropic hormone and analogues
      e.g. corticotropin and tetracosactide.

II. Drugs used in excess of ACTH
   1. CRH-1 receptor antagonists
      e.g. antalarmin and pexacerfont.
   2. Dopamine Agonists
      e.g. bromocriptine and pergolide.
   3. Mono-amine oxidase inhibitors
      e.g. selegiline.
   4. Antihistamines
      e.g. cyproheptadine.

I. Drugs Used in Deficiency of ACTH

1. Adrenocorticotropic Hormone and Analogues

Corticotropin

Corticotropin or Adrenocorticotropic hormone (ACTH) is available commercially and is prepared mainly from porcine pituitaries. It is available as corticotropin (ACTH) for injection, repository corticotropin (ACTH-gel) for injection, and corticotropin zinc hydroxide suspension. Currently, corticotropin is used mainly as a diagnostic tool to assess adrenocortical function.

Pharmacological effects

Exogenous administration of corticotropin essentially produces effects similar to those of endogenous ACTH released from the anterior pituitary gland. It stimulates the production and release of glucocorticoids (mainly hydrocortisone in mammals). At high doses, exogenous corticotropin causes a number of metabolic changes including lipolysis in fat cells, hypoglycaemia and amino acid uptake in muscles.

Pharmacokinetics

Corticotropin is destroyed in the GI tract hence it has to be given parenterally. After IM injection, corticotropin is absorbed rapidly, whereas repository corticotropin injection is absorbed slowly over 8-16 hours. In cattle, it causes increase in secretion of 17-hydroxy corticosteroid within 30 minutes that lasts for about

8-24 hours. The elimination half-life of circulating corticotropin is about 15 minutes, but repository corticotropin (ACTH-gel) preparation gives an extended effect (up to 24 hours). Its short half-life is due to rapid enzymatic hydrolysis.

Side effects/Adverse effects

Side/Adverse effects of exogenous corticotropin are primarily due to excessive secretion of glucocorticoids from the adrenal cortex. Corticotropin isolated from animal pituitary contains significant amount of vasopressin, which can lead to hyponatraemia. Rarely, hypersensitivity reactions can be seen with corticotropin. Prolonged use may result in fluid and electrolyte disturbances and other adverse effects. When used for diagnostic purposes, acute inadvertent over doses of corticotropin are unlikely to cause any significant adverse effects.

Contraindications and precautions

Corticotropin should not be used in patients hypersensitive to porcine proteins. Its use during pregnancy should be avoided. ACTH-gel preparation should not be administered by IV route.

Drug interactions

Prolonged administration of corticotropin shows interactions with a number of drugs including oestrogens, diuretics, oral anticoagulants, barbiturates and cyclophosphamide. These interactions are shown mainly by glucocorticoids secreted by corticotropin administration.

Clinical uses

Naturally obtained corticotropin has limited therapeutic use in both veterinary and human medicines because all of the known therapeutic effects can be achieved with appropriate doses of corticosteroids at a lesser risk of side effects. However, some veterinarians prefer corticotropin over the corticosteroids, especially long-acting synthetic analogues, because exogenous glucocorticoids cause pituitary and adrenal suppression more readily than those produced by corticotropin. In veterinary medicine, corticotropin can be used for stimulation of adrenal cortex when there is deficiency of ACTH and as a therapeutic agent in bovine ketosis.

At present, the major clinical use of corticotropin is in testing the integrity of the hypothalamus-pituitary axis and in the diagnosis of types of adrenal insufficiency. Parenteral injection of corticotropin produces a large increase in hydrocortisone secretion in secondary adrenal insufficiency (i.e. anterior pituitary dysfunction), whereas little or no increase in hydrocortisone secretion occurs in primary adrenal insufficiency (i.e. primary hypoadrenocorticism or Addison's disease). The corticotropin stimulation test is used also to diagnose a variety of adrenopathic disorders including endogenous or iatrogenic hyperadrenocorticism and spontaneous hypoadrenocorticism. An excessive elevation of plasma-hydrocortisone concentration following administration of corticotropin indicates endogenous hyperadrenocorticism, whereas failure of the plasma-hydrocortisone concentration to increase following corticotropin administration indicates either iatrogenic hyperadrenocorticism (exogenous glucocorticoid administration) or spontaneous hypoadrenocorticism. The test, however, does not distinguish spontaneous hypoadrenocorticism from iatrogenic disease. The corticotropin is also used clinically to monitor the response to mitotane (an adrenocorticolytic drug) therapy in Cushing's disease. Nowadays, improved assays for corticotropin are preferred over corticotropin-stimulation test for diagnosis of various adrenocorticopathic disorders.

Dose

For ACTH deficiency/Primary bovine ketosis

Cattle : 200 – 600 Units, IM followed by a daily dose of 200 units.

ACTH-stimulation test (For ACTH-gel preparation)

Dogs & cats : 2.2 Units/kg, IM. Blood samples should be collected for hydrocortisone determination before and at 60 and 10 minutes after injection.

Horses : 1 Unit/kg, IM. Blood samples should be collected for hydrocortisone determination before and at 8 hours after injection.

Tetracosactide

Tetracosactide (Tetracosactrin, cosyntropin, ACTH 1-24) is a synthetic peptide that contains 1-24 N-terminal amino acids of natural ACTH. Its mechanism of action and pharmacological effects are similar to those produced by natural ACTH. It is less immunogenic than the corticotropin as the immunogenicity resides mainly in the 15 amino acids at C-terminal end. Moreover, synthetic tetracosactide does not contain any vasopressin, so it has no effect on salt and water balance. Tetracosactide is safe when used as recommended. It is used mainly to diagnose clinical conditions as mentioned under corticotropin and is preferred over animal derived corticotropin. Similar to corticotropin stimulation test, tetracosactide test involves administration of required drug and estimation of hydrocortisone level before and after injection.

Dose

For Tetracosactide stimulation test

Dogs : 0.125 – 0.25 mg (total), IM or IV.

Cats : 0.125 mg (total), IV.

Horses : 1 mg (total), IV.

II. Drugs Used in Excess of ACTH

Secondary hyperadrenocorticism due to adenoma of the pituitary gland with over secretion of ACTH is a relatively common endocrine disease in dogs. Although the pharmacological treatment of pituitary dependent hyperadrenocorticism is done mainly by the use of adrenocorticolytic drug mitotane (see Chapter 51), several drugs can inhibit excess secretion of ACTH from the pituitary and can be useful in secondary hyperadrenocorticism.

1. Corticotropin Releasing Hormone Receptor Antagonists

Corticotropin receptor antagonists are newly developed group of drugs which block CRH receptor, thereby blocking the consequent secretions of ACTH and hydrocortisone. The main research into CRF antagonists to date has focused on antagonists selective for the CRF-1 subtype, which are expressed only in brain.

Antalarmin

Antalarmin is a non-peptide drug that selectively blocks the CRH-1 receptor. By blocking the CRH-1 receptor, antalarmin reduces the release of ACTH. Excessive release of CRH and ACTH is believed to be directly or indirectly involved in many of the chronic stressful conditions such as stomach ulcers, anxiety, diabetes mellitus, osteoporosis, depression and hypertension. Antalarmin has shown encouraging results in the treatment of anxiety and stress-induced hypertension. Chronic antalarmin

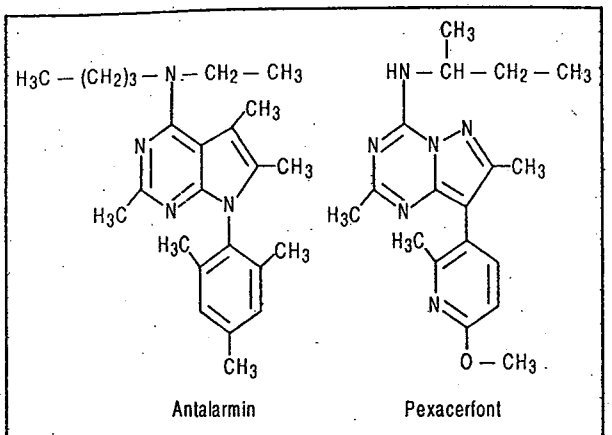


Fig. 49.9. Chemical structures of selected corticotropin releasing hormone receptor antagonists.

treatment also showed anti-inflammatory effects and has been suggested as having potential uses in the treatment of inflammatory conditions such as arthritis, stress-induced gastrointestinal ulcers and irritable bowel syndrome. The drug is presently undergoing clinical trials.

Pexacerfont

Pexacerfont is a recently developed CRF-1 antagonist with activity similar to that of antalarmin. It reduces action of CRH on its receptor and thus decreases synthesis and release of ACTH from anterior pituitary. Similar to antalarmin, pexacerfont is currently undergoing clinical trials for the treatment of anxiety disorders and has also been proposed for the treatment of depression and irritable bowel syndrome.

2. Dopamine Agonists

Bromocriptine

Bromocriptine is a potent dopamine agonist, which may decrease secretion of ACTH through an inhibitory effect on the anterior pituitary. The effect of bromocriptine, however, is variable and is observed only in a small percentage of animals suffering from pituitary-dependent hyperadrenocorticism. Further, relapses of ACTH secretion are common after withdrawal of drug. The pharmacology of bromocriptine has been discussed earlier in the present chapter.

Pergolide

Pergolide is an ergoline-based dopamine receptor agonist, used mainly in treatment of parkinsonian disease in humans. It activates both D_1 and D_2 receptors and is about 10 times more potent than bromocriptine. It also stimulates some serotonin receptor subtypes. In veterinary practice, it has been used in the treatment of pituitary-dependent hyperadrenocorticism primarily to decrease ACTH secretion in horse. However, its response is variable. Anorexia, dyspnoea, sweating, depression and behavioural changes are important side effects related to pergolide. Pergolide has also been shown to impair associative learning.

Dose

Horses : 0.5 mg (total), PO, once daily; dose may be increased gradually to 3 mg once daily.

3. Mono-Amine Oxidase Inhibitors

Selegiline

Selegiline (Deprenyl) is a selective and irreversible inhibitor of monoamine oxidase-B (MAO-B) enzyme. It inhibits ACTH secretion by increasing dopaminergic tone of the pituitary axis. It helps to restore depleted central dopamine levels mainly by selective irreversible inhibition of MAO-B, the enzyme involved in the degradation of dopamine and other catecholamines. Although the effectiveness of selegiline therapy is variable in pituitary dependent hyperadrenocorticism, it is safe and effective drug for the medical management of uncomplicated cases of secondary hyperadrenocorticism in dogs. The pharmacology of selegiline has been described in chapter 23.

Dose

For pituitary dependent hyperadrenocorticism

Dogs : 1 mg/kg, PO, once daily for 2 months. If no improvement is seen, dose may be increased to 2 mg/kg, once daily. If again no improvement is seen or if signs increase, drug should be withdrawn and alternate therapy may be considered.

4. Antihistamines

Cyproheptadine

Cyproheptadine is an antihistamine with serotonin receptor antagonist and calcium-channel blocking properties (see for detail, Chapter 27). The action of cyproheptadine in pituitary dependent hyperadrenocorticism is unclear, although antagonism of serotonin has been suggested. Its effects to decrease the secretion of ACTH are variable and are observed only in small percentage (<10%) of affected animals. Cyproheptadine is used mainly as adjunctive therapy for Cushing's syndrome.

Dose

For hyperadrenocorticism

Dogs : 0.3 mg/kg, PO, increasing gradually to 3 mg/kg daily.

POSTERIOR PITUITARY HORMONES

The posterior pituitary (neurohypophysis) secretes two major hormones – vasopressin (antidiuretic hormone) and oxytocin. Unlike anterior pituitary hormones which are synthesised by pituitary cells, vasopressin and oxytocin are synthesised by the supraoptic (mainly vasopressin) and paraventricular nuclei (mainly oxytocin) of the hypothalamus and then passed down the axons in combination with carrier proteins called neurophysins into the posterior pituitary. In the posterior pituitary, the hormones are stored in many secretory granules present in the dilated nerve endings and then released into adjacent capillaries by exocytosis.

VASOPRESSIN/ANTIDIURETIC HORMONE

Vasopressin (Antidiuretic hormone, argipressin) is a posterior pituitary hormone that is synthesised by neurons situated primarily in the supraoptic nuclei of the hypothalamus. It is involved mainly in the regulation of water balance, but also has an important pressor effect. It plays a key role in homeostasis and in regulation of water, glucose and salts in the blood.

Chemistry

Natural vasopressin is a nonapeptide with a sulphydryl bridge connecting residues 1 and 6, which is necessary for the physiological activity. Reduction of the disulphide inactivates the

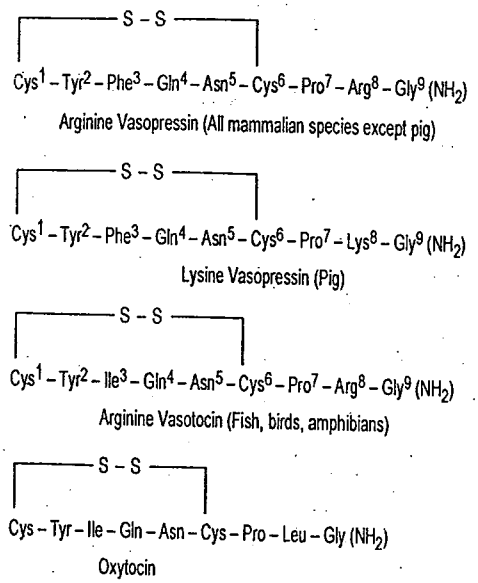


Fig. 49.10. Chemical structures of hormones of posterior pituitary gland.

hormone. Residue 8 is arginine in all species, except pigs in which it is lysine. Therefore, the main form of vasopressin in man, dog and most species is arginine vasopressin, whereas in pigs it is lysine arginine (Figure 49.10). Lysin vasopressin has only about half the antidiuretic activity of arginine vasopressin. Vasopressin in birds contains isoleucine in place of phenylalanine at position 3 of arginine vasopressin and is called arginine vasotocin.

Regulation of secretion

In contrast to hormones of the anterior pituitary, vasopressin (also oxytocin) is not regulated by any hypothalamic releasing hormone. The output of vasopressin is directly related to the degree of hydration of body and an increase in plasma osmolality is the most important physiological stimulus for vasopressin release. Whenever the plasma osmolality increases above a threshold level (>280 mOsm/kg), selective osmoreceptors located in the hypothalamus are stimulated which in turn activate cells responsible for the synthesis and secretion of vasopressin. Release of vasopressin causes increased solute-free water reabsorption, thereby maintaining plasma osmolality and solute/water ratio within a few percent of the normal values. Increased osmolality also stimulates receptors in oesophagus and stomach which increase desire for water intake (thirst). Reduction in effective blood volume and/or arterial blood pressure also increases vasopressin release mainly through activation of stretch receptors located in atria and baroreceptors in carotid sinus and aortic arch (Figure 49.11). Increase in renin and angiotensin production in response to hypovolaemic/hypotension also releases vasopressin and produces a sensation of thirst.

In addition to enhanced osmolality, hypotension and/or hypovolaemia, several endogenous hormones and neurotransmitters modify vasopressin secretion by stimulating or inhibiting neurons involved in its synthesis and release. Acetylcholine (via nicotinic receptors), histamine, dopamine, glutamine, aspartate, prostaglandins, neuropeptide Y and substance P stimulate vasopressin release, whereas GABA, opioids (mainly dynorphin via κ-receptors) and arterial natriuretic peptide decrease vasopressin release. Pain, nausea and hypoxia also stimulate vasopressin secretion. Drugs like epinephrine, antidepressants, cyclophosphamide and nicotine stimulates vasopressin release, whereas ethanol, phenytoin, glucocorticoids, haloperidol, promazine, butorphanol and low doses of morphine suppress vasopressin secretion.

Mechanism of action

The vasopressin produces its cellular effects mainly by interacting with two main types of G-protein coupled receptors – V_1 and V_2.

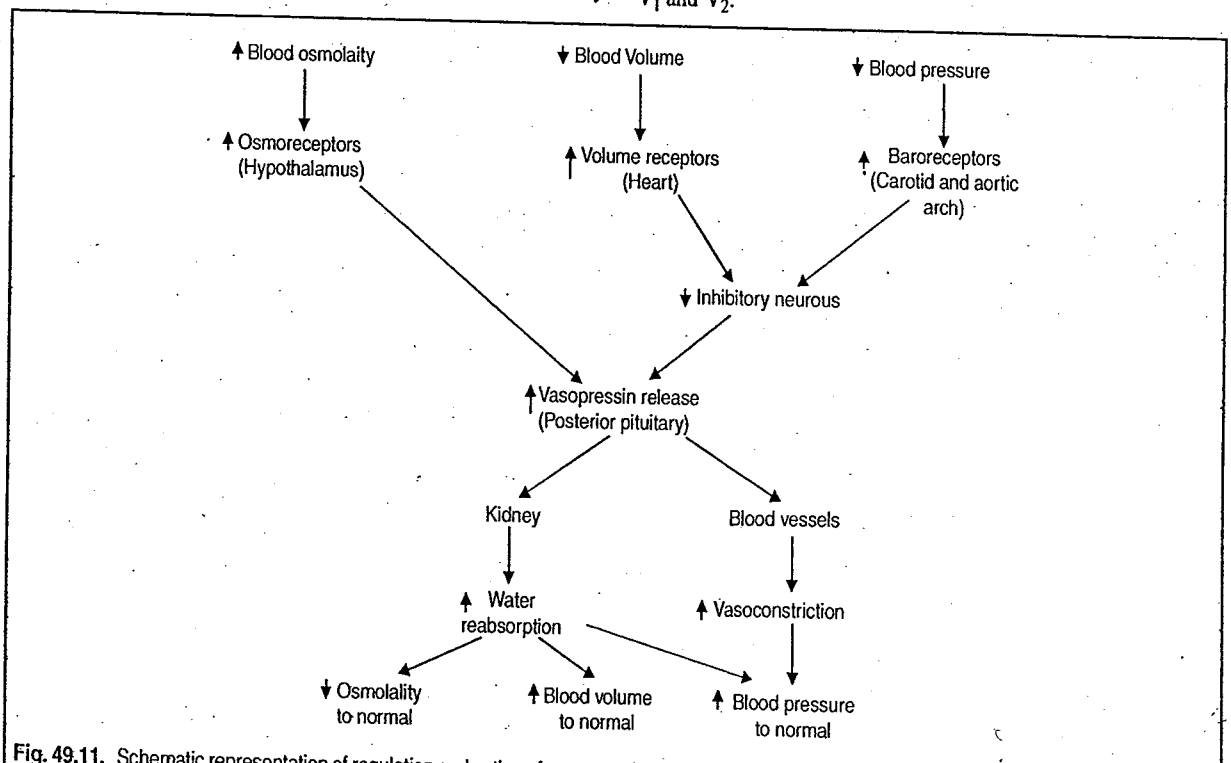


Fig. 49.11. Schematic representation of regulation and action of vasopressin

V_1-Receptor: The V_1-receptors, also called arginine vasopressin-1 (AVP_1) receptors, have been sub classified further as V_{1A} (AVP_{1A}) and V_{1B} (AVP_{1B}) receptors. The V_{1A} is widely distributed in vascular smooth muscle, myocardium, urinary bladder, hepatocytes, platelets, testes, adipocytes, CNS and kidneys (renal medullary interstitial cells in renal cortical collecting ducts). The V_{1B} receptors are located only in the adenohypophysis. Both V_{1A} and V_{1B} are coupled to inositol phosphate production.

Vasopressin binds to V_1 receptors and activates phospholipase enzymes (PLC, PLD and PLA_2). Activation of phospholipase C, probably via G_q G-protein, causes generation of inosistol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) through hydrolysis of phosphatidylinositol-4,5-bisphosphate (Figure 49.12). Enhanced IP_3 in turn leads to increased intracellular calcium concentration either through release of Ca^{++} from its intracellular storage sites or by enhancing influx of extracellular Ca^{++} via Ca^{++} channels located on the cell membrane. Ca^{++} binds to and activates a number of intracellular proteins which lead to the ultimate cellular response i.e. vasoconstriction, platelet aggregation, glycogenolysis, ACTH release and growth of vascular smooth muscle cells. Stimulation of phospholipase D by V_1 receptors also mediates the enhanced synthesis of DAG through hydrolysis of other phospholipids. Activation of protein kinase C by DAG leads to the phosphorylation of key enzymes, which contribute to the biological response of vasopressin. Lastly, stimulation of phospholipase A_2 mobilises arachidonic acid from membrane phospholipids. Arachidonic acid in turn is metabolised via cyclooxygenase and epoxygenase to various prostaglandins and epoxyeicosatrienoic acid, respectively, which modulate biological responses (Figure 49.12).

V_2-Receptor: The V_2 receptors, also called arginine vasopressin-2 (AVP_2) receptors are present mainly in the principal cells of renal collecting duct system and have also been detected in some blood vessels. The V_2 receptors are more sensitive to vasopressin than V_1 receptors and are coupled to adenylate cyclase enzyme through G_s G-protein. Activation of V_2 receptors located on the basolateral membrane of principal cells of renal collecting ducts (CD) leads to enhanced intracellular cAMP via adenylate cyclase enzyme. The intracellular cyclic AMP, in turn activates cAMP dependent protein kinase A that through phosphorylation of relevant proteins promotes exocytosis of water channel-containing vesicles (WCVs) into the apical membrane and also a decreased rate of endocytosis of WCVs from the apical membrane (Figure 49.12). This leads to insertion of more water channels (also called aquaporin-CD) into the apical membrane at the same time with decreased removal and degradation of water channels. This allows water molecules to flow down a concentration gradient from an area of higher concentration in the renal filtrate to an area of lower concentration in the interstitium of the renal medulla. Activation of V_2 receptors also increases

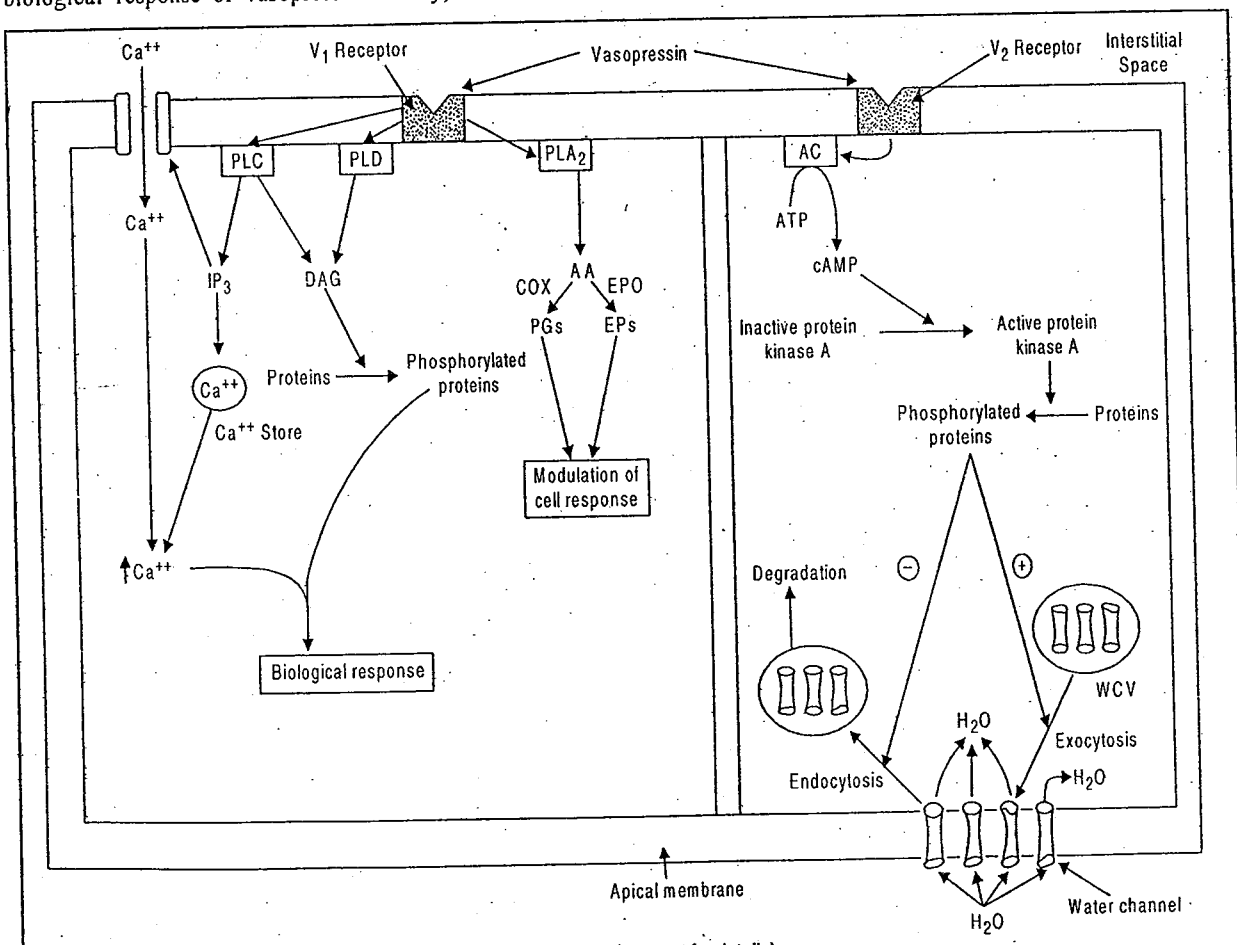


Fig. 49.12. Schematic representation of mechanisms of vasopressin (see text for details).
Abbreviations : PLC = phospholipase C ; PLD = phospholipase D ; PLA_2 = phospholipase A_2 ; IP_3 = 1,4,5-inositol triphosphate ; DAG = 1,2-diacylglycerol ; V_1 and V_2 = vasopressin receptors ; AA = arachidonic acid ; PGs = prostaglandins ; EPO = epoxygenase; EPs = epoxyeicosatrienoic acid ; COX = cyclooxygenase; AC = adenylate cyclase ; ATP = adenosine triphosphate ; cAMP = cyclic adenosine monophosphate ; WCV = water channel containing vesicles; H_2O = water; + = stimulation ; – = inhibition.

urea permeability of the terminal part of the collecting ducts probably by an analogues action on urea channels.

Physiological effects

Vasopressin has both renal and extra-renal effects.

Renal effects : Vasopressin has an important antidiuretic effect. In kidneys, it binds to V_2 receptors to increase water permeability and resorption in the collecting duct tubules. Up to 90% of the fluid filtered by glomerulus and not absorbed in proximal nephron is reabsorbed under the influence of vasopressin. In the absence of vasopressin, collecting duct cells remain impermeable to water and dilute urine is passed as such. It also transiently increases sodium absorption. The V_1 receptors participate in the renal response to vasopressin by modulating glomerular filtration, blood flow through vasa recta, prostaglandins production from interstitial cells and reactivity of collecting duct cells to V_2 receptor stimulation. In general, the V_1 action tends to restrain V_2 mediated water permeability. Since V_2 action is produced at much lower concentration of vasopressin, V_1 renal actions may serve to restrict V_2 effects when blood levels of vasopressin are very high.

Extra-renal effects:

Cardiovascular system: Vasopressin causes constriction of vascular smooth muscle by acting on V_{1A}-receptor and produces rise in blood pressure. Blood vessels of the skin, mesenteric, skeletal muscle, thyroid, fat and pancreas appear to be more sensitive to the action of vasopressin than the blood vessels present in the GI tract, heart and CNS. However the effects of vasopressin on blood vessels are seen only at high concentrations because the sensitivity of V_1 receptors is lesser than the sensitivity of renal V_2 receptors. Therefore under physiological conditions, the effect of vasopressin on blood vessels is not considered much important. Prolonged exposure to vasopressin causes vascular smooth muscle hypertrophy. A vasodilatory action of V_2 receptor has also been reported in some blood vessels, but is seen only when a V_1 antagonist or a selective V_2 agonist is used.

Non-vascular smooth muscle: At high concentration, vasopressin stimulates uterine (via oxytocin receptors) and gastrointestinal (via V_1 receptors) smooth muscles. In non-pregnant and in early pregnancy, the vasopressin induced uterus contraction is almost equipotent to oxytocin. Only at term, sensitivity to oxytocin increases selectively.

Central nervous system: Vasopressin has been found to act as a central neuromodulator and neurotransmitter. It can modulate CNS autonomic system controlling arterial blood pressure, heart rate and respiration rate, but the physiological significance of these actions is not clear. Further, vasopressin is involved also in the release of ACTH, although it is not the principal corticotropin-releasing hormone.

Blood coagulation: Vasopressin stimulates aggregation and degranulation of platelets by an action on V_1-receptors and increases the concentration of Factor VIII of coagulation cascade by an action on V_2-receptor.

Other effects: Vasopressin accelerates glycogen breakdown in liver cells. It has antipyretic activity as it is reported to function as a modulator of fever, when released endogenously.

DISORDERS OF VASOPRESSIN

Deficiency of vasopressin

Deficiency of vasopressin leads to production of large amount of dilute urine, a syndrome called diabetes insipidus. Diabetes insipidus may be hypothalamic (central) or nephrogenic. Hypothalamic diabetes insipidus results from inadequate secretion of vasopressin, whereas nephrogenic diabetes insipidus results from abnormalities in kidneys which prevent them from responding to vasopressin. The hypothalamic form of central diabetes insipidus develops as a result of compression or destruction of the cells responsible for synthesis and release of vasopressin. It may occur because of large pituitary neoplasm, a dorsally expanding cyst or inflammatory granuloma, traumatic injury to skull with haemorrhage and glial proliferation in the neurohypophyseal system. Affected animals excrete large volumes of hypotonic urine and drink equally large volume of water. Diabetes insipidus occurs infrequently in dogs and cats and rarely in other species.

Excess of vasopressin

Hypersecretion of vasopressin in the absence of osmotic or volumetric stimulation leads to a disease called 'Syndrome of inappropriate antidiuretic hormone (SIADH)'. The SIADH results from impaired water excretion with accompanying hyponatraemia and hypoosmolality. It may occur due to a number of factors including malignancy, pulmonary disease, CNS injury or disease, (e.g. head trauma, infections and tumours), general surgery and pharmacological agents (e.g. cyclophosphamide, thiazide diuretics, tricyclic antidepressants, nicotine and vinca alkaloids). In a normal animal, an elevation of plasma vasopressin levels does not induce plasma hypotonicity because the animal simply stops drinking water due to an osmotically induced aversion to fluids. Hypersecretion of vasopressin results in hypotonicity of plasma only when it is accompanied with excessive fluid intake (oral or IV). The clinical manifestations of plasma hypotonicity resulting from SIADH in man include lethargy, anorexia, nausea/vomiting, muscle cramps, coma, convulsions and death. SIADH has been reported in dogs, but the incidence and importance of this problem in other animal species is uncertain.

TREATMENT OF VASOPRESSIN DISORDERS

Drugs either activating or inhibiting the activity of vasopressin are available for clinical use. These drugs are used mainly in human medicine for several conditions. Some of these drugs are available also for use in domestic animals.

Classification

Drug affecting the vasopressin action are broadly divided into two categories: drugs used in deficiency of vasopressin and drugs used in excess of vasopressin.

I. Drugs used in deficiency of vasopressin
   1. Vasopressin and analogues
      e.g. vasopressin, desmopressin, terlipressin and felypressin.
   2. Miscellaneous drugs
      e.g. chlorpropamide, clofibrate, carbamazepine and thiazides.

II. Drugs used in excess of vasopressin
   1. Vasopressin receptor antagonists/Vaptans
   a. Non-selective vasopressin receptor antagonists
      e.g. conivaptan.
   b. Selective V_1 vasopressin receptor antagonists
      e.g. relcovaptan and nelivaptan.

c. Selective V_2 vasopressin receptor antagonists
e.g. tolvaptan, mozavaptan, lixivaptan and satavaptan.

2. Tetracyclines
e.g. demeclocycline.

I. Drugs Used in Deficiency of Vasopressin

1. Vasopressin and Analogous

Vasopressin

Vasopressin (Antidiuretic hormone) is commercially available both as arginine vasopressin (vasopressin, argipressin, pitressin) and lysine-vasopressin (lypressin), or a combination of arginine and lysine vasopressins. These products are derived either from natural sources (cattle or swine pituitary) or prepared synthetically. The products are standardised by their pressor activity in rats and expressed in Units (USP units). Lysine vasopressin has only about half the antidiuretic activity of arginine vasopressin. Commercially available vasopressin has little, if any, oxytocic activity at usual doses.

Pharmacological effects

The mechanism of action and pharmacological effects of exogenous vasopressin administration are similar to those of endogenous vasopressin released from the posterior pituitary. It promotes renal reabsorption of solute free-water in the distal convoluted tubules and collecting duct by increasing water permeability at the luminal surface. Vasopressin causes increase in urine osmolality and decrease in urine flow.

In high doses, vasopressin produces vasoconstriction, especially in arterioles and capillaries, and elevates blood pressure. It decreases blood flow to several organs, but hepatic blood flow can be increased. It stimulates contraction of smooth muscle of GI tract and can increase peristalsis, particularly of the colon. It also increases GI sphincter pressure, but decreases gastric secretions; gastric acid concentration remains unchanged. At large doses, vasopressin has oxytocin like activity and may cause contraction of uterus. It may also stimulate contractions of gall bladder and urinary bladder smooth muscles and causes release of ACTH, growth hormone and FSH.

Pharmacokinetics

Like other peptide hormones, vasopressin is ineffective when given orally because it is rapidly destroyed in GI tract. After IM or SC administration, vasopressin is distributed throughout the extracellular fluid. Inactivation by peptidases in various tissues (mainly liver and kidneys) results in a plasma half-life of vasopressin of only few minutes. In dogs, aqueous vasopressin has diuretic activity for 2-8 hours. Vasopressin tannate injection (Pitressin tannate in oil) is a longer acting preparation, which is repeated every 1 to 3 days.

Side effects/Adverse effects

Important adverse effects related to vasopressin include skin reactions, nausea, abdominal pain, pallor, urge to defecate, local irritation at injection site and rarely a hypersensitivity reaction (urticaria). Overdosage may produce fluid retention, hyponatraemia, arrhythmias, listlessness and depression. Very high doses may lead to severe water intoxication with clinical signs of coma, seizures and eventually death.

Treatment: Treatment of water intoxication includes discontinuation of vasopressin therapy and water restriction. Severe intoxication may require the use of osmotic diuretic (e.g. mannitol, urea or dextrose) with or without furosemide.

Contraindications and precautions

Vasopressin is contraindicated in patients suffering from chronic nephritis, cardiac disease, hypertension and those are hypersensitive to it. It should not be used in patients with seizure disorders and in pregnant animals.

Drug interactions

Vasopressin shows interactions with a number of drugs. Large doses of epinephrine, alcohol, heparin and demeclocycline may inhibit the activity of vasopressin, while urea, carbamazepine and chlorpropamide may potentiate the effects of vasopressin. Drugs which inhibit prostaglandins synthesis may facilitate action of vasopressin. Lithium inhibits the effect of vasopressin.

Clinical uses

In veterinary medicine, vasopressin is used occasionally in the treatment of central (vasopressin responsive) diabetes insipidus and as a diagnostic agent in small animals. Aqueous vasopressin is not suitable for long-term management of diabetes insipidus because its duration of action is only few hours. Further, with difficult availability of vasopressin tannate in oil and wider acceptance of desmopressin, the therapeutic utility of vasopressin in the treatment of central diabetes insipidus has declined. In human medicine, vasopressin is also used occasionally to treat acute GI haemorrhage and to stimulate GI peristalsis. Vasopressin often stops bleeding by constriction of mesenteric blood vessels and reducing blood flow, allowing clot formation.

As a diagnostic agent, vasopressin is used in the differential diagnosis of diabetes insipidus i.e. to distinguish between the central form of the disease from the nephrogenic form. The vasopressin/ADH response test is performed after a water-deprivation test to see if the diabetic animals can concentrate urine or not. A marked response (i.e restoration of the ability to concentrate urine) confirms central diabetes insipidus, whereas failure to respond to vasopressin indicates presence of nephrogenic diabetes insipidus. Failure to respond to vasopressin indicates also that treatment with vasopressin or analogues in nephrogenic diabetes will not be successful. Water deprivation test should not be conducted in animals those are dehydrated or suffering from renal disease.

Dose

For treatment of central (ADH-responsive) diabetes insipidus

Vasopressin tannate in oil

Dogs : 2.5 – 5 Units, IM, every 1 to 7 days.

For Diagnosis (vasopressin response test)

Dogs & cats : 0.5 unit/kg (maximum 5 units), IM. Urine samples should be collected 2 hourly following the injection until maximum concentration is achieved.

Desmopressin

Desmopressin acetate is a synthetic analogue of arginine-vasopressin, which interacts primarily with the V_2 receptors. It is used in the treatment of central diabetes insipidus and is particularly indicated when the disease is severe. Currently, desmopressin is preferred over vasopressin for the treatment of central diabetes insipidus.

Chemistry

Desmopressin is 1-deamino-(8-D-arginine)-vasopressin. Substitution of D-arginine for L-arginine at 8 amino acid greatly reduces vasopressor activity without reducing diuretic activity, and deamination at position 1 increases duration of action and increases antidiuretic activity.

Pharmacological effects

Desmopressin acts primarily on V_2-receptor and increases water reabsorption by the collecting ducts in kidney. It increases urine osmolality and decreases net urine production. Therapeutic doses do not directly affect urinary sodium or potassium excretion. Desmopressin does not possess the vasoconstrictor activity of vasopressin. The antidiuretic to vasopressor ratio for desmopressin is approximately 3000-fold greater than that of vasopressin. Similar to vasopressin, it increases circulating levels of pro-coagulant Factor VIII and von Willebrand factor through activation of vasopressin receptors in endothelial cells. Although desmopressin has no direct effect on blood vessels, fall in blood pressure may occur as a result of deceased renin and angiotensin (renal effects) in the body.

Pharmacokinetics

Desmopressin is inactivated in GI tract (oral bioavailability ~10%); hence it must be given either parenterally or topically. The onset of antidiuretic action in dogs after nasal or sub-conjunctival administration usually occurs within one hour, peak effect occurs from 2 to 8 hours, and duration of action varies from 8 to 24 hours. In man, the plasma half-life of desmopressin has two components, a fast component of 6.5 to 9 minutes and a slow component of 30 to 120 minutes.

Side effects/Adverse effects

Side/Adverse effect with therapeutic doses of desmopressin is not common. Occasionally, nasal or eye irritation may occur after topical application. Rarely, hypersensitivity reactions may occur. Excessive desmopressin medication can lead to hyponatraemia and water intoxication. Clinical signs may include depression salivation, nausea, vomiting, ataxia, muscle tremors, convulsion and coma.

Treatment: Overdosage of desmopressin may be treated by water restriction and dosage reduction/stoppage of desmopressin therapy.

Contraindications and precautions

Desmopressin is contraindicated in patients which are hypersensitive to it or for the treatment of type IIB or platelet-type (pseudo) von Willebrand's disease because it may cause platelet aggregation and thrombocytopenia. It should be used with caution in patients suffering from renal disorders, in pregnancy and coagulation disorders. The dose should be adjusted to the requirements of the individual patient. For animals those are markedly polyuric and polydipsic, access to water should be limited immediately after therapy to avoid subcellular overhydration.

Drug interactions

Drug interactions of desmopressin are similar to those of vasopressin.

Clinical uses

In veterinary and human medicines, desmopressin is used mainly in the treatment of central diabetes insipidus and in the differential diagnosis of diabetes insipidus to differentiate between the central and nephrogenic form of the disease (See for detail vasopressin). It is also used therapeutically for short-term management or prevention (preoperatively) of bleeding in patients suffering from a deficiency of von Willebrand factor. However, development of resistance, short duration of activity and high cost limits its usefulness for this bleeding disorder. In human medicine, desmopressin nasal spray at bedtime controls primary nocturnal (bed-wetting) in children by reducing urine volume.

Currently desmopressin is available as a product approved for use in human in three preparations - parenteral injection, nasal drops and oral tablets. In human medicine, intranasal administration is commonly used, but in animals topical administration to the conjunctiva is preferred. Ophthalmic route of administration is preferred because repeated intranasal use in animals often proves difficult. Daily administration of desmopressin may be limited because of the expense of the product.

Dose

For therapeutic purposes

For treatment of central diabetes insipidus

Dogs & cats : 2 – 4 drops, subconjunctivally (of intranasal solution desmopressin 0.1 mg/ml), 1 to 2 times daily.
: 0.2 mg (total), PO, 1 to 3 times daily.
: 0.001 – 0.004 mg (total), IM, 1 to 2 times daily.

For treatment of von Willebrand's disease

Dogs & cats : 0.001 mg/kg, IV (if patient is bleeding), or IV or SC (90 minutes before surgery as a preventive measure).
: 0.1 – 0.2 mg (total), PO.

For diagnostic purposes (vasopressin response test)

Dogs & cats : 0.002 to 0.004 mg (total), IM. Urine samples should be collected 2 hourly following the injection until maximum concentration is achieved.

Cats : 1 drop subconjunctivally (of intranasal solution), 2 times daily for 2 to 3 days. A marked reduction in water intake or a 50% or greater increase in urine reduction suggests a deficit in vasopressin production (central diabetes insipidus).

Terlipressin

Terlipressin is the triglycyl derivative of lysine-vasopressin, having predominant action on V_1-receptor. It possesses a low but protracted vasopressor activity, with minimal antidiuretic properties. In human medicine, terlipressin is used in the treatment of bleeding oesophageal varices. It has a prolonged action, is simple to use and is more effective than vasopressin. It is usually categorised as an orphan drug.

Other drugs

Some other drugs possessing activity similar to that of vasopressin include felypressin and ornipressin. Felypressin (Phe^2-Lys^8-vasopressin) is an analogue of lysine vasopressin. It is predominantly vasoconstrictor in action and is added to some

local anaesthetics such as prilocaine. Ornipressin is a vasoconstrictor, haemostatic and renal agent.

2. Miscellaneous Drugs

Several drugs which are structurally unrelated to vasopressin are used occasionally in the treatment of partial or complete central diabetes insipidus.

Chlorpropamide

Chlorpropamide is an oral sulphonylurea hypoglycaemic drug that has been used in the treatment of partial central diabetes insipidus. It is thought to act by potentiating the renal tubular effects of endogenous vasopressin. In human patients, chlorpropamide decreases urine output 30% to 70% at 3 to 10 days of therapy. Success in dogs is variable, ranging from about 20% to 50% reduction in urine output in 1 to 2 weeks of medication. Hypoglycaemia is a potent side effect, which can be minimised by regular feeding. The pharmacology of chlorpropamide is given in Chapter 53.

Dose

Dogs : 10 – 40 mg/kg, PO, 1 to 2 times daily.

Cats : 50 mg (total), PO, once daily.

Clofibrate

Clofibrate is a hypolipidaemic agent that appears to cause release of vasopressin from the hypothalamus. It is used in human medicine for controlling the high cholesterol and triacylglyceride levels in the blood. It acts by increasing the lipoprotein lipase activity to promote the conversion of very-low-density lipoprotein (VLDL) to low-density lipoprotein (LDL). However, its clinical efficacy as hypolipidaemic agent is unpredictable. Excessive use of clofibrate has been associated with induction of SIADH, the syndrome of inappropriate secretion of antidiuretic hormone.

Carbamazepine

Carbamazepine is an anticonvulsant drug that appears to stimulate release of vasopressin from the hypothalamus and increase tubule response to the hormone. High doses are needed for the desirable effect. It is of little value in treatment of diabetes insipidus due to marked adverse effects. The pharmacology of carbamazepine has been discussed earlier in Chapter 22.

Thiazides

Thiazide diuretics such as chlorothiazide and hydrochlorothiazide paradoxically can reduce urine output in animals with diabetes insipidus. They do not affect vasopressin in the body but act primarily by total body salt depletion with subsequent contraction of the extracellular fluid compartment.

I. Drugs Used in Excess of Vasopressin

1. Vasopressin Receptor Antagonists /Vaptans

The vasopressin receptor antagonists, often called vaptans, are a novel group of pharmaceuticals which antagonize action of vasopressin on various vasopressin receptor subtypes. The group contains a number of compounds with varying selectivity, several of which are either already in clinical use or in clinical trials.

a. Non-selective Vasopressin Receptor Antagonists

Conivaptan

Conivaptan is a non-selective vasopressin receptor antagonist with actions on V_{1A} and V_2 vasopressin receptors. It was approved in 2004 for treatment of hyponatraemia caused by syndrome of inappropriate antidiuretic hormone (SIADH). In addition, conivaptan has been tried for the treatment of decompensated congestive heart failure. The safety and efficacy of conivaptan for the treatment of congestive heart failure has not been fully established. The pharmacology of conivaptan is given in Chapter 39.

b. Selective V_1-Vasopressin Receptor Antagonists

Relcovaptan

Relcovaptan is a non-peptide vasopressin receptor antagonist with selective action at the V_{1A} subtype. It has shown significant uterine relaxant effects in pregnant women. It has also shown positive initial results in tests against Raynaud's disease and dysmenorrhoea. The drug is presently undergoing clinical trials.

Nelivaptan

Nelivaptan is a selective and orally active non-peptide vasopressin receptor antagonist selective for the V_{1B} subtype. Currently it is undergoing clinical trials for the treatment of anxiety and depression.

c. Selective V_2-Vasopressin Receptor Antagonists

Tolvaptan

Tolvaptan is selective vasopressin V_2 receptor antagonist. Early animal studies demonstrated that tolvaptan is about 30 times more selective for V_2 receptors than for V_{1A} receptors. Currently it is being marketed for treatment of hyponatraemia associated with congestive heart failure, cirrhosis and syndrome of inappropriate antidiuretic hormone (SIADH) due to ADH producing tumours. Tolvaptan is generally well tolerated with most frequently reported adverse effects of thirst and dry mouth. The pharmacology of tolvaptan is discussed also in Chapter 39.

Mozavaptan

Mozavaptan is a vasopressin receptor antagonist with selective action on V_2 receptors. Its pharmacological properties are similar to those of tolvaptan. It has been approved for treatment of hyponatraemia caused by syndrome of inappropriate antidiuretic hormone (SIADH) due to ADH producing tumours.

Other drugs

Lixivaptan and satavaptan are two other selective vasopressin V_2 receptor antagonists. These drugs are currently not available for clinical use, but are in clinical trials for treatment of hyponatraemia and the signs and symptoms of water retention associated with heart failure and syndrome of inappropriate antidiuretic hormone.

2. Tetracyclines

Demeclocycline

Demeclocycline, a tetracycline antibiotic, is sometimes used to block the action of vasopressin in the kidney in hyponatraemia due to SIADH, when fluid restriction has failed. Action of demeclocycline in excess of vasopressin secretion is only symptomatic and non-selective. The use of demeclocycline in SIADH actually relies on one of its side effects i.e. demeclocycline induces nephrogenic diabetes insipidus. Earlier, it was the only drug available for treatment of SIADH, but now has been superseded by newly introduced vasopressin receptor antagonists, such as tolvaptan.

OXYTOCIN

Oxytocin (Greek word *oxytocic* = rapid birth) is a polypeptide hormone that is produced in neuronal bodies in the paraventricular (mainly) and supraoptic nuclei of the hypothalamus and then stored and released in the posterior pituitary. Oxytocin, unlike vasopressin, acts selectively on the smooth muscle cells of the uterus and the myoepithelial cells of mammary gland and is involved mainly in parturition and milk let down.

Chemistry and synthesis

Oxytocin is a nonapeptide and is structurally similar to vasopressin except that in oxytocin isoleucine and leucine replace phenylalanine and arginine of vasopressin molecule at positions 3 and 8, respectively. Like vasopressin, the S-S bond of cystine is crucial for its activity as the biological activity is completely lost if it is reduced. Oxytocin has a molecular weight of 1007 Da.

The synthesis of oxytocin, as described previously for protein and peptide hormones, involves first the production of a pre-prohormone called pre-prooxyphysin at the level of cell bodies within the hypothalamus. The pre-portion of the molecule is cleaved to form pro-oxyphysin, which in turn is cleaved to form oxytocin. Transport, storage and release of oxytocin are similar to those descried for vasopressin. Oxytocin is also synthesised and secreted by the corpus luteum of ewes, cattle and primates. Oxytocin has a short life of few minutes.

Regulation of release

Release of oxytocin from posterior pituitary is regulated by sensory stimuli arising from the cervix and vagina and suckling/milking reflex from the mammary gland. Stimulation of the cervix in a pregnant animal elicits nervous signals which pass to the hypothalamus and cause increased secretion of oxytocin. The amount of oxytocin in plasma increases during labour, especially during the last stage. Similarly, the suckling stimuli on the nipple/teat of mammary gland transmit signals through sensory nerves to the oxytocin neurons in hypothalamus to cause release of oxytocin from posterior pituitary gland (Figure 49.13). In less than a minute after beginning of suckling, milk begins to flow (milk letdown or milk ejection). In addition to suckling reflex, manual stimulation of teat washing before milking or any other sensory stimulus that an animal associates with milking gives signal to hypothalamus to cause milk letdown.

The release of oxytocin from posterior pituitary is controlled by adrenergic and cholinergic pathways as well as opioid peptides and oestrogen. Opiates appear to block secretion of oxytocin by binding to opioid receptors present in the posterior pituitary. Ovarian relaxin also blocks oxytocin. Many psychogenic factors or generalised sympathetic stimulation can inhibit oxytocin secretion and consequently depress milk letdown. Similar to vasopressin, secretion of oxytocin is provoked by increases in osmolality of plasma and is suppressed by ethanol. Many factors which cause release of vasopressin also release oxytocin, and factors which release oxytocin can release vasopressin probably due to their identical structure, synthesis, storage and release. However, the proportion of oxytocin to vasopressin release can vary depending on the nature of stimulus.

Plasma oxytocin levels may also vary during the reproductive cycle. In cattle, sheep and goats, plasma levels of oxytocin increase after the preovulatory LH surge and decrease at the time of luteal regression. Ovarian oestrogen may enhance oxytocin release, whereas progesterone inhibits it. In male animals, mating may be a stimulatory factor.

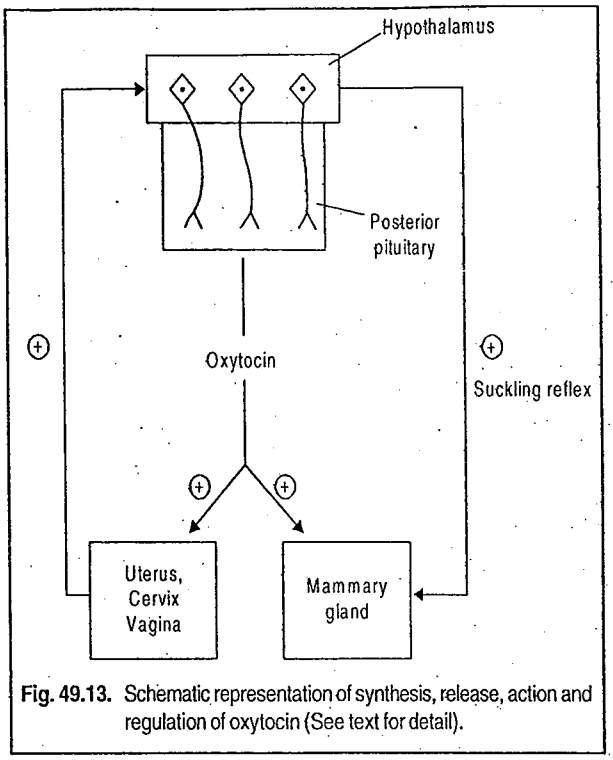


Fig. 49.13. Schematic representation of synthesis, release, action and regulation of oxytocin (See text for detail).

Mechanism of action

Oxytocin acts by binding to its specific oxytocin (OXT, OT) receptors present in the myometrium and mammary glands. The OXT receptor protein belongs to the G-protein coupled receptor family, specifically the G_q protein. Oxytocin receptors are expressed by the myoepithelial cells of the mammary gland, and in both the myometrium and endometrium of uterus at the end of pregnancy. In uterus, two distinct receptors subtypes have been identified, which regulate mainly the contractile properties of myometrial cells and elicit prostaglandin production by endometrial/decidual cells. Following binding, oxytocin stimulates smooth muscle contractions and also induces $PGF_{2\alpha}$ release which causes luteolysis. These receptors mediate the response mainly by depolarisation of muscle fibres and influx of Ca^{++} ions as well as through activation of phospholipase C and release of intracellular Ca^{++} by inositol-1,4,5-triphosphate (IP_3). Receptors in the mammary gland increase during pregnancy and remain constant during lactation. In myometrium, the number of oxytocin receptors increases just prior to parturition mainly under the influence of oestrogen and decreases thereafter. Progesterone priming of the myometrium for a period appears essential before oestrogen can induce myometrial oxytocin receptors.

Physiological effects

Oxytocin is best known for roles in female reproduction with action on uterus and mammary glands.

Uterus: Oxytocin increases both the force and frequency of pregnant uterine muscles, especially towards end of gestation. These effects are highly dependent on the presence of oestrogen. The increased contractility is restricted to the fundus and body; lower segment is not contracted or may even be relaxed at term. Immature uterus, non-pregnant uterus and uterus during early

pregnancy are quite resistant to physiological concentration of oxytocin (exogenous oxytocin can increase or enhance rhythmic contractions at any time).

Mammary gland: Oxytocin acts on myometrial cells which surround outer walls of the alveoli and cause letdown of milk. In this process, oxytocin causes contraction of myometrial cells and forces milk from the alveoli into the ducts and cisterns, so that young one can obtain it by suckling. Although the milk is synthesised and secreted continuously into alveoli of the mammary gland under the influence of various hormones like prolactin, oestrogen and progesterone, its removal requires oxytocin.

Oxytocin is synthesized by corpora lutea of several species, including ruminants and primates. Along with oestrogen, it is involved in inducing the endometrial synthesis of prostaglandin $F_{2\alpha}$ to cause regression of the corpus luteum. Recent studies have suggested role of oxytocin in various types of behaviour, including social recognition, pair bonding, anxiety and maternal behaviours. In male animals, oxytocin has no physiological functions, although it is suggested that oxytocin facilitates sperm transport through the male reproductive tract.

DISORDERS OF OXYTOCIN

No pituitary dysfunction affecting oxytocin release in domestic animals has been reported so far. However, the inefficient milk let down reflex leading to decrease release of oxytocin from pituitary is an important cause of agalactia in animals.

TREATMENT OF OXYTOCIN-RELATED DISORDERS

Treatment of oxytocin related disorders, in particular deficiency of oxytocin release causing insufficient milk let down, is a common feature in both human and veterinary medicines.

Classification

I. Drugs used in deficiency of oxytocin
   1. Oxytocin and analogues
      e.g. oxytocin, posterior pituitary extract, carbotocin and demoxytocin.

II. Drugs used in excess of oxytocin
   e.g. atosiban.

I. Drugs Used In Deficiency of Oxytocin

1. Oxytocin and Analogues

Oxytocin

Commercially, oxytocin is either extracted from animal posterior pituitary or preferably prepared synthetically. The commercially available preparations are highly purified and have virtually no antidiuretic or vasoconstrictor activity at normal doses. Oxytocin potency is standardised according to its vasodepressor activity in chickens and expressed in posterior pituitary units. One USP unit is equivalent of approximately 2-2.2 micrograms of pure oxytocin.

Pharmacological effects

The mechanism of action and pharmacological effects of exogenous oxytocin at normal doses are essentially similar to those of endogenous oxytocin. It stimulates both frequency and force of contraction of uterine smooth muscle and contracts myoepithelial cells of mammary gland to cause milk letdown. When oxytocin is given by a slow IV infusion, it produces a rapid increase in the tone and motility of uterus at term. The non-pregnant uterus is not as sensitive to oxytocin as pregnant uterus, but it can be stimulated at very high doses. Oxytocin at high doses exerts a vasopressin like action i.e. urine output is decreased and hyponatraemia secondary to free water retention is produced. At high doses, oxytocin also cause vasodilatation by direct action and produces a brief fall in blood pressure. This action is most marked in chicken. In birds, vasopressin is far more active in stimulating contractions of the oviduct than is oxytocin.

Pharmacokinetics

Oxytocin is ineffective orally as proteolytic enzymes in the GI tract degrade it. Therefore, it must be administered parenterally or as a nasal spray. It has a rapid onset of action and a short half-life. Following IM administration, the uterus responds generally within 3-5 minutes and after IV administration the response is immediate. The duration of effect following parenteral injection varies from 10 to 20 minutes. Oxytocin given parenterally does not enter the brain in significant quantities as it fails to cross the blood-brain barrier. Systemic oxytocin is metabolised rapidly in the liver and kidney. During pregnancy, certain oxytocin inactivating enzymes (oxytocinases) appear in the plasma, placenta and uterus and are also considered to be involved in regulating the concentration of oxytocin.

Side effects/Adverse effects

Judicious use of oxytocin at normal doses does not produce significant side/adverse effects. Occasionally swelling and sloughing at the site of injection and rarely hypersensitivity reaction may be observed. Excessive amount of oxytocin may lead to tetanic spasm of uterus, foetal distress and asphyxia, rupture of uterus and cervix, and foetal death. Effects of overdosage on uterus depend on the stage of uterus and the position of foetus(es). Oxytocin overdosage may also produce hypotension and reflex tachycardia. Water intoxication can occur if large doses are infused for a longer period of time, especially if large volume of electrolyte-free intravenous fluids is concomitantly administered. Severe intoxication may produce coma, seizures and death.

Treatment: Treatment of mild oxytocin intoxication includes discontinuation of therapy. Water intoxication may require use of osmotic diuretics with or without furosemide and water restriction till condition is resolved.

Contraindications and precautions

Oxytocin is contraindicated in first and second stages of labour, improper dilation of cervix, closed pyometra, dystocia due to abnormal presentation of foetus(es) unless correction is made, and in patients those are hypersensitive to it. Before using oxytocin, hypoglycaemia or hypocalcaemia should be treated. Indiscriminate use of oxytocin for letdown of milk in dairy animals should be avoided.

Clinical uses

In veterinary medicine, oxytocin is used for induction and enhancement of uterine contractions at parturition, dystocia due to secondary uterine inertia, treatment of postpartum retained placenta and metritis, and in treating agalactia due to failure of milk letdown. It is also used in control of postpartum haemorrhage

and to hasten uterine involution immediately after parturition or after manual correction of prolapsed uterus. Oxytocin administered IV has been used in the treatment of mastitis in cattle as an aid to expressing all infected milk from the quarters prior to introducing chemotherapeutic agents.

Rarely, oxytocin challenge test is used to determine utero-placental adequacy in high-risk pregnancies in human medicine. Oxytocin is infused IV at very low concentration till uterine contractions are elicited every 3-4 minutes. A marked increase in foetal heart rate indicates utero-placental inadequacy. The test is very risky.

Dose

To facilitate uterine contractions during parturition

Dogs	:	1 – 5 units, SC or IM. Dose may be repeated after 30 minutes, if required.
Cattle	:	30 units, IM. Dose may be repeated after 30 minutes, if required.
Horses	:	10 – 40 units, IM.
	:	2.5 – 10 units, slow IV.
Pigs	:	10 units, IM. Dose may be repeated after 30 minutes, if required.

For uterine inertia

Dogs	:	5 – 20 units, IM or IV. Dose may be repeated only after 30 minutes, if required.
Cats	:	2.5 – 5 units, IM or IV.
Cattle & horses:		10 – 40 units, IM.
	:	2.5 – 10 units, slow IV of diluted solution.
Sheep & pigs	:	2 – 10 units, IM.
	:	0.5 – 2.5 units, slow IV.

For letdown of milk/agalactia due to failure of letdown

Dogs	:	Oxytocin nasal spray, 5 to 10 minutes prior to nursing, 3 times daily.
Cattle & horses:		20 – 80 units, IM.
	:	5 – 20 units, slow IV.
Sheep & pigs	:	4 – 20 units, IM.
	:	1 – 5 units, slow IV.

Posterior pituitary extract

Posterior pituitary extract (Pituitary extract-Posterior lobe) is the extract of posterior pituitary gland. It is used occasionally to stimulate contraction of the oestrogen-sensitised myometrium and mammary myoepithelial cells. Activity is expressed as units of oxytocic activity. Its side/adverse effects, contraindications and clinical uses are similar to those of oxytocin. However, it is less efficacious and less potent than pure oxytocin and is less used clinically.

Dose

For uterine inertia/agalactia/uterine involution

Dogs	:	2.5 – 20 units, SC or IM.
Cats	:	2.5 – 5 units, SC or IM.
Cattle & horses:		30 – 100 units, IM or SC.
Pigs	:	10 – 30 units, IM or SC.

Carbotocin

Carbotocin is a synthetic analogue of oxytocin. Its pharmacological effects are similar to those of oxytocin such as contraction of the uterus. It is used in conditions sensitive to oxytocin such as to control postpartum haemorrhage.

Demoxytocin

Demoxytocin is a synthetic analogue of oxytocin. It is used largely in obstetrics for the prevention of puerperal mastitis.

II. Drugs Used in Excess of Oxytocin

Oxytocin Receptor Antagonists

Recently, some oxytocin receptor antagonists like atosiban have been developed, which competitively inhibit the effect of oxytocin. These drugs are being investigated for the prevention of premature labour.

Atosiban

Atosiban is a chemically modified form of oxytocin. It acts on the OXT receptors in the uterus and blocks the contractile effects of oxytocin on myometrium. It also blocks action of vasopressin on smooth muscles, but the effect is seen in slightly higher concentration. Atosiban is mainly used as tocolytic agent to delay premature labour. It is well tolerated at normal doses.

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CHAPTER 50

THYROID AND PARATHYROID HORMONES

Thyroid hormones
- Thyroid gland
- Thyroid hormones
  - Chemistry
  - Synthesis, storage, release, transport, metabolism and excretion
  - Regulation of secretion
  - Mechanism of action
  - Physiological effects
  - Disorders of thyroid hormones
    - Deficiency of thyroid hormone/hypothyroldism
    - Excess of thyroid hormones/hyperthyroidism
    - Goitre/hyperplasia of thyroid follicular cells.
    - Treatment of thyroid hormone disorders
      - Classification
      - Drugs used in deficiency of thyroid hormones
        - Thyroid hormones and analogues
        - Miscellaneous drugs
      - Drugs used in excess of thyroid hormones/ hyperthyroidism
        - Drugs inhibiting thyroid hormone synthesis
        - Drugs destroying thyroid tissue
        - Drugs inhibiting hormone release
        - Drugs inhibiting iodide trapping/ionic inhibitors
        - Miscellaneous drugs

Calcitonin
- Synthesis and secretion
- Regulation of secretion
- Mechanism of action
- Physiological effects
- Disorders of calcitonin
  - Deficiency of calcitonin/Hypocalcitoninism
  - Excess of calcitonin/Hypercalcitoninism
  - Treatment of calcitonin disorders

Parathyroid hormone
- Parathyroid gland
- Parathyroid hormone
  - Synthesis, secretion and metabolism
  - Regulation of secretion
  - Mechanism of action
  - Physiological effects
  - Disorders of parathyroid hormone
    - Deficiency of parathyroid hormone/Hypoparathyroidism
    - Excess of parathyroid hormone/Hyperparathyroidism
  - Treatment of parathyroid hormone disorders
    - Drugs used in deficiency of parathyroid hormone
    - Drugs used in excess of parathyroid hormone
      - Calcitonin and analogues
      - Vitamin D analogues

THYROID HORMONES

Thyroid Gland

The thyroid gland is an important endocrine gland required for metabolic regulation and various physiological processes. It secretes 3 hormones – thyroxine (T_4), triiodothyronine (T_3) and calcitonin. The T_4 and T_3 hormones are produced by thyroid follicles and are termed as thyroid hormones. Calcitonin produced by interfollicular 'C' cells is chemically and biologically different from T_4 and T_3 and it regulates calcium metabolism.

The thyroid gland is present in all vertebrates. In most mammals, thyroid gland is composed of two lobes lying on either side of the larynx and upper trachea and connected by a narrow piece of tissue called the isthmus. In birds, thyroid gland is present within the thoracic cavity with both lobes near the syrinx adjacent to carotid artery. Ectopic or accessory thyroid tissue is relatively common in most species, especially dogs and cats, and can be located anywhere from the larynx to the diaphragm. Accessory thyroid tissue can maintain normal thyroid function after surgical thyroidectomy and occasionally is the site of hyperplasia and neoplasia.

The thyroid gland is composed of large number of closed follicles (100 to 300 μm in diameter), the functional units of the thyroid. Each follicle consists of a single layer of epithelial cells around the cavity called the follicle lumen, which is filled by a thick secretory substance called colloid. The major constituent of colloid is the large glycoprotein thyroglobulin, which contains the thyroid hormones within its molecule. The follicular cells are cuboidal when the secretion is basal and elongated when cells are stimulated to release hormones. The paracellular or C-cells are located outside the follicles and secretes calcitonin.

Thyroid Hormones

The thyroid hormones include two iodine containing hormones, the thyroxine (T_4) and triiodothyronine (T_3). Both hormones are synthesised and stored in the thyroid follicles and are critically important for the normal growth and development, and energy metabolism.

Chemistry

The thyroid hormones T_4 and T_3 are iodine-containing hormones and are unique in that they are the only iodinated organic compounds in the body. Both T_4 and T_3 are derivatives of thyronine, which is a condensation product of two molecules of the amino acid tyrosine. Thyroxine is 3,5,3',5'-tetraiodothyronine, while T_3 is 3,5,3'-triiodothyronine. Both hormones exist within the molecules of thyroglobulin, a 660 kDa glycoproteins synthesised and secreted by thyroid cells.

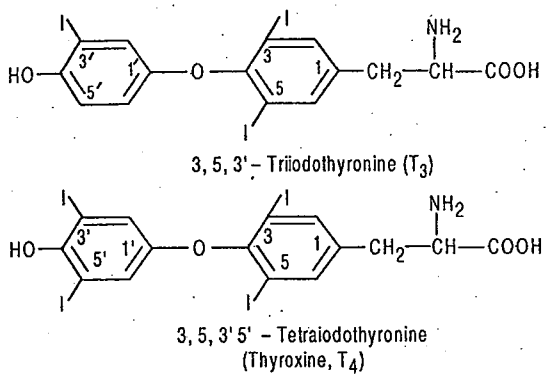


Fig. 50.1. Chemical structures of thyroid hormones.

Synthesis, storage, release, transport, metabolism and excretion

Synthesis and storage: The biosynthesis of thyroid hormones is unique among endocrine glands because the final assembly of hormones occurs extracellularly within the follicular lumen. Two molecules viz. iodine and tyrosine are essentially required for thyroid hormone synthesis. Tyrosine is present as a part of the glycoprotein thyroglobulin, each molecule of which contains 115 tyrosine residues. Iodide is trapped by follicular cells from the plasma, transported rapidly against a concentration gradient to the lumen and oxidised to iodine in the microvilli membranes. The synthesis involves several steps, which have been summarized below and depicted in Figure 50.2.

a. ***Iodide uptake:*** Iodides ingested orally are absorbed from the GI tract into blood in about the same manner as chlorides. Most of the absorbed iodide is excreted rapidly by kidneys, but about one fifth is selectively removed by the glandular and follicular cells of the thyroid gland. Although the concentration of iodide in blood is low (0.2 – 0.4 µg/dl in humans), thyroid cells have a specific active transport process called iodide pump or sodium-iodide symporter, which pumps iodide from outside into interior of the cell by a process known as 'iodide trapping'. In this process, sodium is co-transported with iodide from the basolateral side of the membrane into the cell. The iodide trapping is stimulated by thyroid stimulating hormone (TSH) and also is controlled by an autoregulatory mechanism. This allows intracellular iodide concentration to be 25 to 200 times greater than extracellular levels. Although the iodide trapping mechanism is present also in some other organs like skin, salivary gland, gastric mucosa, mammary gland and placenta, but it is not stimulated by TSH.

b. ***Oxidation and iodination:*** The iodide trapping by follicular cells is oxidised by a heme-containing thyroid peroxidase enzyme (thyroperoxidase) to iodinium ion (I^+), hypoiodous acid (HOI) or enzyme linked hypoiodate (E-OI). The peroxidase enzyme is located at or near the apical membrane and utilises hydrogen peroxide (H_2O_2) as the oxidant. When the thyroglobulin molecule is secreted through apical membrane into follicles, the oxidised iodide binds avidly with about one sixth of tyrosine amino acid within the thyroglobulin molecule. Tyrosine is iodinated apparently without any enzymatic intervention; first at position 3 on the ring to form monoiodotyrosine (MIT) and then, in some molecules, on position 5 as well forming diiodotyrosine (DIT), while these residues are still attached to the thyroglobulin chain. Two of these molecules are then coupled, either MIT with DIT to form T_3 or two DIT molecules to form T_4 (Figure 50.3). Normally much more T_4 than T_3 is formed, but during iodine deficiency relatively more MIT is available and greater proportion of T_3 is formed. The mechanism of coupling is not fully known but is believed to involve the same thyroid peroxidase system that is involved in iodination. Finally, the coupling between two adjacent thyroglobulin molecules occurs that results in the final stored follicular thyroglobulin having molecular weight twice of that originally secreted thyroglobulin. Oxidation of iodine and coupling are both stimulated by TSH.

c. ***Synthesis of thyroglobulin:*** Thyroglobulin is a high molecular weight (600,000 to 750,000) glycoprotein synthesised in successive subunits on the ribosomes of endoplasmic reticulum in follicular cells. The constituent amino acids (tyrosine and others) and carbohydrates (e.g. mannoses, fructose and galactose) come from the circulation. Recently synthesised thyroglobulin leaves the Golgi apparatus and is packaged into apical vesicles and extruded into the follicular lumen. Iodine is bound to tyrosil residues in thyroglobulin usually at apical surface of follicular cells to form successively MIT and DIT. Each thyroglobulin molecule contains 1-3 T_4 molecules and an average of 1 T_3 molecule for every 14 molecules of T_4.

d. ***Storage of thyroglobulin:*** After coupling, thyroglobulin containing iodinated tyrosil and thyronil residues is transported to interior of follicles and remains stored as thyroid colloid in the lumen until released. This extracellular storage of hormone allows the thyroid gland to have a large reserve of thyroid hormones sufficient for normal requirements of body for 2 to 3 months. Therefore, when synthesis of thyroid hormone ceases, the effects of deficiency are not observed for several months.

Release: The release of thyroid hormones is initiated by endocytosis of thyroglobulin from the follicular lumen at apical surface of the cell. This starts with formation of pseudopods, which engulf some of colloid in the lumen. The endocytic vesicles then fuse with the lysosomes to form digestive vesicles which contain requisite digestive enzymes. The proteinases (proteolytic enzymes) among these enzymes digest the thyroglobulin molecules and release T_4 and T_3, which then diffuse into the surrounding capillaries (Figure 50.2). The MIT and DIT which fail to become thyroid hormones (about three quarters of the iodinated tyrosine in thyroglobulin never become thyroid hormones under normal

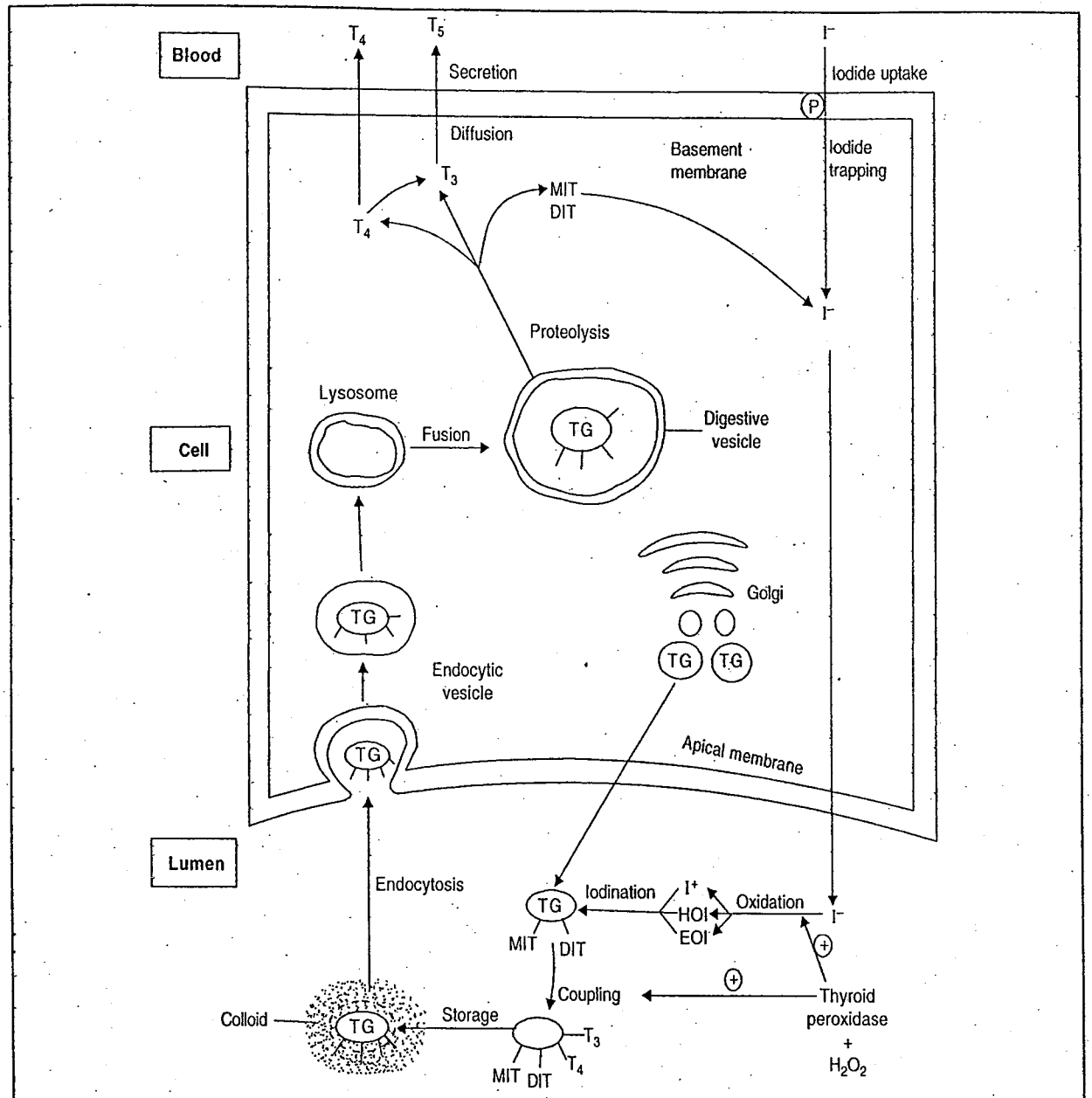


Fig. 50.2. Schematic representation of synthesis, storage and secretion of thyroid hormones (See text for detail).
Abbreviations : I^- = iodide ; I^+ = iodinium ion ; HOI = hypoiodous acid ; EOI = enzyme linked hypoiodate ; H_2O_2 = hydrogen peroxide; TG = thyroglobulin ; MIT = monoiodotyrosine ; DIT = diiodotyrosin; P = iodide pump, T_3 = Triiodothyronine ; T_4 = thyroxine; + = stimulation.

conditions) are also released at the same time. However, they are not secreted into blood but are metabolised (deiodinated) within the cell and the iodide is reused. TSH appears to enhance the degradation of thyroglobulin by increasing activity of several endopeptidases of the lysosomes.

Transport: The major form of thyroid hormone in the blood is thyroxine (T_4), which has a longer half life than T_3. The ratio of T_4 to T_3 released into the blood is roughly 20 to 1. On entering blood, the thyroid hormones combine immediately with several plasma proteins including thyroxine-binding globulin (TBG), thyroxine binding pre-albumin and albumin. The most important carrier protein in all domestic animals, except cat, is TBG that has high affinity for T_4 (also T_3), but low capacity because of its low concentration. Albumin has low affinity, but high capacity for T_4 and T_3 because of its high concentration in plasma. The pre-albumin has intermediate affinity and is specific for T_4. Because of high lipid solubility and high affinity for plasma proteins, more than 99% of the thyroid hormones are present in bound form and less than 1% is available for action. However, the equilibrium between free and bound forms of hormone is easily shifted depending on the physiological or pharmacological situations such as increased concentration of oestrogen that occurs during pregnancy or enhanced synthesis of thyroid hormones under influence of TSH.

Both thyroid hormones, in particular thyroxine, are released slowly to the tissue cells. On entering the tissue cells both hormones again bind with intracellular proteins, thyroxine again binding strongly than the triiodothyronine. In the functional tissues, they are used slowly over a period of days and weeks.

Metabolism and excretion: In the peripheral tissues, mainly liver, kidneys and skeletal muscle, T_4 is metabolised by mono-deiodination to form two main metabolites viz. 3,5,3'-

triiodothyronine (T_3) and 3,3',5'-triiodothyronine (reverse T_3, rT_3). About one-third of T_4 secreted by thyroid undergoes conversion to T_3 that is the main hormone regulating energy metabolism, and about 40% is converted to reverse T_3 (Figure 50.3) which is inactive and that may inhibit the calorigenic actions of T_3. Most of the T_3 in plasma is derived from liver produced by the bioactivation process. Target tissues take up T_3 from circulation for the metabolic need, except brain and pituitary which take up T_4 and convert it to T_3 within their own cells. Therefore, it is suggested that T_3 is the main active hormone, while T_4 largely acts as a prohormone.

The thyroid hormones (both T_4 and T_3) are eventually degraded by deiodination, deamination, decarboxylation and conjugation with glucuronide and sulphate mainly in the liver. The free and conjugated forms are excreted partly in bile and partly in urine. Degradation of the conjugated forms in intestine results in production of iodide molecules, which are reabsorbed as part of the enterohepatic cycle. In humans, the metabolic clearance of T_3 ($t½$ = 1 day) is 20 times faster than that of T_4 ($t½$ = 6-7 days). One reason for these long half-lives of thyroid hormones (in contrast to other hormones which have short lives of seconds to minutes) is their high affinity for plasma proteins, which protects them from degradation. However, some domestic species have a relatively short half-life of thyroid hormones with dogs exhibiting the plasma half-lives of T_4 and T_3 of 12-24 hours and 6 hours, respectively.

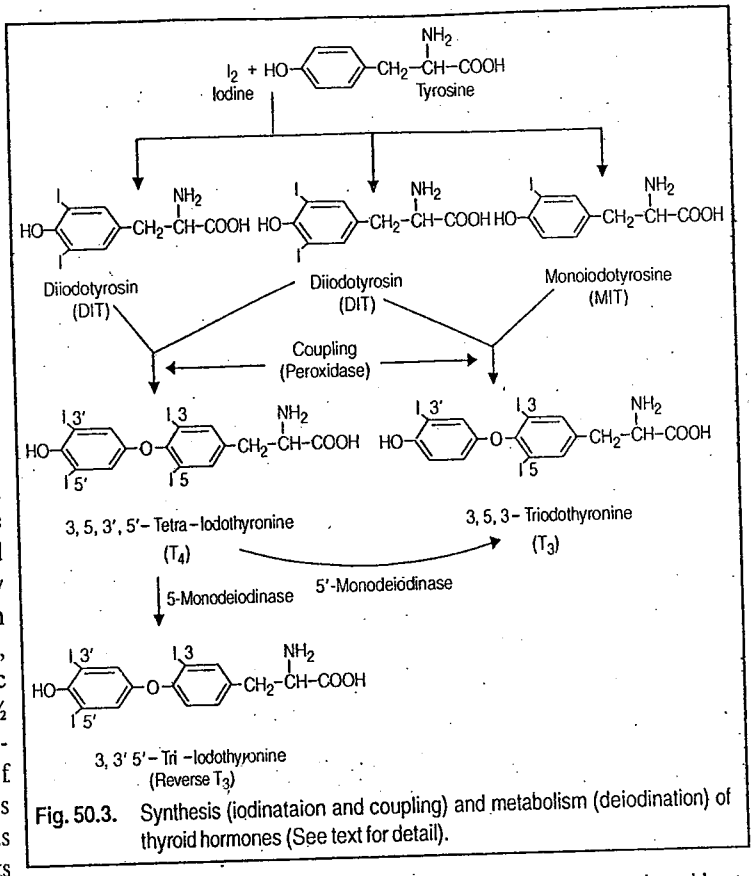


Fig. 50.3. Synthesis (iodinataion and coupling) and metabolism (deiodination) of thyroid hormones (See text for detail).

Regulation of Secretion

The secretion of thyroid hormones from thyroid glands is controlled mainly by the anterior pituitary by elaboration of thyroid stimulating hormone (TSH) or thyrotropin (see Chapter 49). The TSH released from the thyrotroph cells of anterior pituitary combines with the follicular cell membrane and stimulates both thyroid hormones synthesis and secretion. In general, TSH stimulates almost all aspects of thyroid hormone synthesis and release i.e. iodide uptake by follicle cells, oxidation and coupling processes, generation of hydrogen peroxide and iodination of tyrosine, blood flow through gland, synthesis of thyroglobulin, and endocytosis and proteolysis of thyroglobulin. The production of TSH, in turn is influenced by a negative feedback effect of thyroid hormones, T_3 being more active than T_4. The extent of conversion of T_4 to T_3 and its degradation in tissues affect plasma concentrations of thyroid hormones and thus influence the negative feedback effect on TSH release.

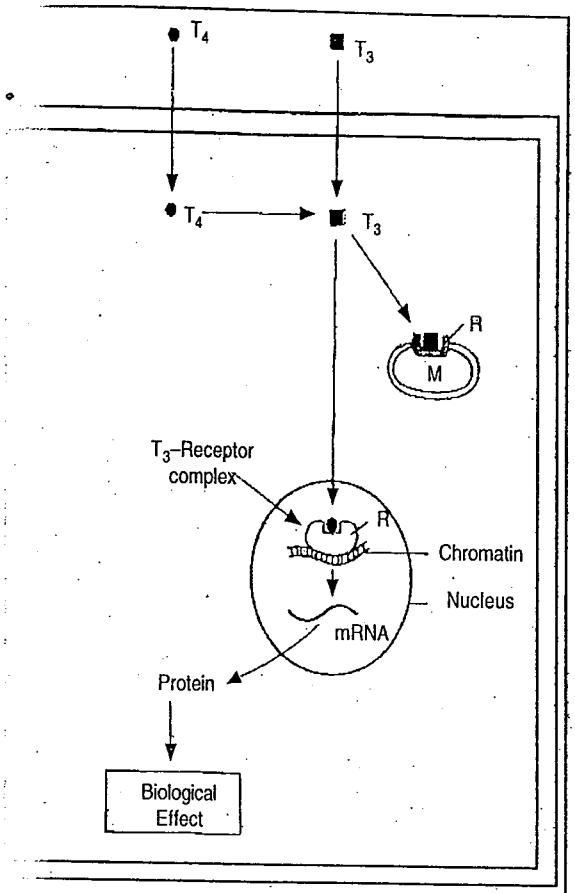
The other main factor influencing thyroid function is the plasma iodide concentration. A reduced plasma iodide concentration results in decrease of hormone production and an increase in TSH secretion; an increased plasma iodide has the opposite effect. However, the overall feedback mechanism responds to changes of iodide only slowly, over fairly long periods (days to weeks). A prolonged decrease of iodine in the diet results in a continuous excessive secretion of TSH and eventually in an increase in vascularity and hypertrophy of the thyroid gland.

Other factors which affect thyroid function include somatostatin, a hypothalamic secretion, which reduces basal TSH secretion. Various anions such as nitrate, thiocyanate and perchlorate inhibit sodium iodide uptake by competing with iodide for active transport in the follicular cells. Thioureylene drugs (e.g. thiamazole) inhibit oxidation and coupling processes. Propranolol (high doses), amiodarone, propylthiouracil and glucocorticoids inhibit peripheral conversion of T_4 to T_3, except in brain and pituitary.

Mechanism of action

Thyroid hormones are lipophilic compounds, which penetrate the cell membrane easily even in a passive manner. However, at least 10 different active, energy dependent and genetic regulated iodothyronine transporters have also been identified. After they enter cell, most of the T_4 is converted to T_3, which binds with high affinity to a well-studied set of nuclear receptors associated with DNA in the nucleus of the cell, called the thyroid hormone receptors (Figure 50.4). A specific DNA sequence call 'thyroid hormone response element' has been identified in the regulatory region of specific genes to which the T_3-receptor complex binds. The affinity of receptor for T_4 is less by a factor of 4-10; so only small amounts of T_4 are bound. Although the precise biochemical mechanism through which thyroid hormones exert their developmental and tissue-specific effects are not clearly understood, the hormones probably act by a mechanism similar to that of steroids and modulates gene transcription and ultimately protein synthesis. It is believed that most, it not all, of the actions of thyroid hormone result from the enzymatic and other functions of hundreds of new types of proteins (e.g. structural proteins, transport proteins and growth proteins).

Apart from the nuclear thyroid receptor, several other sites of thyroid hormone action have been identified. Both T_4 and T_3 act on cell membrane to enhance amino acids and glucose entry. Free T_3 in cell binds to high affinity receptors on the inner mitochondrial (MT) membrane and activates oxidative



3.4. Schematic representation of proposed subcellular mode of thyroid hormone action (see text for detail).
eviations : T_3 = Triiodothyronine ; T_4 = thyroxin, R = thyroid one receptor ; mRNA = messenger ribonucleic acid ; itochondrion.

horylation. Sensitisation of adrenergic receptors to olamines probably by induction of adenylate cyclase e and proliferation of β-adrenoceptors and a better coupling en these have also been documented.

logical effects

he thyroid hormones affect functions of practically all body therefore, their physiological effects are wide and difficult fine precisely. The overall functions of T_3 and T_4 are similar, gh much of the biological activity is the result of T_3 only. ly, the physiological effects of thyroid hormones can be ed into two main categories – those affecting growth and opment and those affecting metabolism. In addition, effects rious systems are observed.

Growth and development: Thyroid hormones (both T_3 and e essentially required for normal growth and development. affect growth and development partly by a direct action on and partly indirectly by influencing growth hormone tion and potentiating its effects. The thyroid hormones are larly necessary for normal growth and maturation of CNS, efore birth and after birth. If the foetus does not secrete ent quantities of thyroid hormone, growth and maturation in are greatly retarded and the brain remains smaller than . In humans, absence of thyroid hormones during the active enesis (up to 5 months post-partum) leads to irreversible retardation (cretinism) and is accompanied by multiple logical alterations in brain due to disturbed neuronal migration, deranged axonal projection and decreased synaptogenesis.

In addition to CNS, thyroid hormones affect growth and development in a large number of tissues by promoting protein synthesis and enzymatic activity. Myelin basic protein, a major component of myelin, is regulated by thyroid hormone during development. Thyroid hormones act in concert with growth hormone and affect pre-pubertal growth. The thyroid hormones are also important for a normal response to parathormone and calcitonin and for skeletal development.

Metabolism: Thyroid hormones regulate metabolism in most tissues, T_3 being 3 to 5 times more active than T_4. Thyroid hormones have marked effect on basal metabolic rate and carbohydrate, lipid and protein metabolism. Most of their actions involve modulation of other hormones and endogenous substances. Thyroid hormones activity is usually defined in terms of tissue or organ and response to inadequate or excessive amount of the hormone.

Basal metabolic rate and calorigenesis: Thyroid hormones are considered as primary determinant of the basal metabolism and stimulate basal metabolic rate (BMR), increase oxygen utilization and heat production by almost all cells of the body. Excessive amount of thyroid hormones can increase the BMR to 60 to 100 percent above normal, whereas their deficiency can reduce the BMR to 30 to 50 percent below normal. Thyroid hormones stimulate cellular metabolism and produce energy; excess energy being released as heat. The mechanism of calorigenic is believed to be uncoupling of oxidative phosphorylation; however, other processes may also be involved. Although most of body tissues are affected by thyroid hormones, the metabolic rate in some tissues like gonads, brain, spleen, uterus and lymph nodes are affected less. The heart, kidneys, liver and muscle are affected most by thyroid hormones.

Carbohydrate metabolism: Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhanced glucose absorption from GI tract, rapid uptake of glucose by cells, enhanced glycolysis, enhanced gluconeogenesis and increased insulin secretion. These effects probably result from the overall increase in cellular metabolic enzymes caused by thyroid hormones.

Lipid metabolism: Thyroid hormones stimulate essentially all aspects of fat metabolism. They accelerate all phases of cholesterol metabolism and enhance lipolysis. In particular, lipid is mobilised from fat tissues and fat is depleted, which increases free fatty acid concentration in plasma but decreases plasma concentration of cholesterol, phospholipids and triglycerides. They also greatly accelerate the oxidation of free fatty acids by cells. The lipolytic action of thyroid hormones is mediated mainly indirectly through catecholamines and other lipolytic hormones and accumulation of cAMP through inhibition of phosphodiesterase enzyme. Conversely, decreased thyroid secretion greatly increases plasma concentrations of cholesterol, phospholipids and triglycerides and cause excessive accumulation of fat in liver.

Protein metabolism: Thyroid hormones increase synthesis of certain proteins, but overall effect is catabolic with increased amount of protein being used as energy source. Prolonged action of thyroid hormones usually results in negative nitrogen balance and tissue wasting. Greatly increased thyroid hormones decrease the body weight, whereas greatly decreased hormones increase the body weight.

Vitamin metabolism: Because vitamins form an essential part of some enzymes and coenzymes and are required essentially for various metabolic processes, enhanced metabolic rate induced by thyroid hormones may produce a relative vitamin deficiency in the body.

Effect on body systems

Cardiovascular system: Thyroid hormones increase heart rate and force of contraction, mainly indirectly through their interactions with catecholamines and up-regulation of beta-adrenoceptors as well as through direct action of contractile elements. Blood pressure is elevated because of increased systolic pressure with no change in diastolic pressure, the end result being an increase in cardiac output. The thyroid hormones cause vasodilatation in most of body tissues and increase blood flow. The rate of blood flow to skin is especially increased because of increased need for heat elimination. Thyroid hormones cause blood volume to increase slightly partly from vasodilatation, which allows increased quantities of blood to collect in the circulatory system.

Respiratory system: The increased rate of metabolism increases utilisation of oxygen and formation of carbon dioxide. These mechanisms increase the rate and depth of respiration.

Gastrointestinal tract: Thyroid hormones increase appetite and food intake. They also increase the rate of secretion of digestive juices and propulsive activity of GI tract. Diarrhoea often results in hyperthyroid patients, while constipation is common in hypothyroidism.

Skeletal muscles: Slight increase in thyroid hormones usually increases muscle activity, but in higher concentration of hormones muscles become weak because of excess protein catabolism. Deficiency of thyroid hormones makes muscles sluggish, which relax slowly after a contraction.

Reproduction: Thyroid hormones are required for normal sexual activity and maintenance of pregnancy and lactation. In men, lack of thyroid hormones causes loss of libido, while excess of hormones frequently causes impotence The effect of thyroid hormones on reproduction result mainly from the altered metabolic and inhibitory feedback effects operating through the anterior pituitary hormones.

DISORDERS OF THYROID HORMONES

Deficiency of Thyroid Hormone/Hypothyroidism

An inability of thyroid gland to secrete adequate amounts of thyroid hormones leads to a condition called hypothyroidism. The hypothyroidism is most common in dogs but also develops rarely in other species including cats and large domestic animals. Thyroid deficiencies are known under various names including hairless calves, hairless pigs, big neck, goitre and myxoedema. Although dysfunction anywhere in the hypothalamic-pituitary axis may result in thyroid hormone deficiency, more than 95% of the clinical cases of hypothyroidism results from the destruction of thyroid gland itself called the primary hypothyroidism. Important causes of primary hypothyroidism include lymphocytic thyroiditis, idiopathic follicular atrophy and neoplastic destruction of thyroid tissues. Lymphocytic thyroiditis in dogs closely resembles Hashimoto's disease in humans and appears to be genetically conditioned through production of autoantibodies directed against thyroglobulins. In idiopathic follicular atrophy or follicular collapse, there is progressive loss of follicular epithelium and replacement of adipose connective tissue with a minimal inflammatory response. Congenital absence of thyroid gland, deficiency to iodine, surgical thyroidectomy and ingestion of goitrogenic plants (e.g. cabbage, linseed, clover and rapeseed) and drugs (e.g. sulphonamides, para-aminosalicylic acid, phenylbutazone and chlorpromazine) are rarer causes of hypothyroidism in small animals. Secondary hypothyroidism, resulting from a deficiency of thyroid stimulating hormone (TSH) and tertiary hypothyroidism resulting from lack of hypothalamic thyrotropin releasing hormone (TRH) are less prevalent causes of hypothyroidism.

Hypothyroidism in dogs is most common in mid to large size breeds around the age of 4-10 years. The clinical signs of hypothyroidism refer to multiple body systems; as a result manifestations are diffuse, variable, often non-specific and rarely pathognomonic. Clinical disturbance vary among affected animals and not all signs are seen in each patient. Clinical signs are related directly to slowing of basal metabolic rate and include mental dullness, lethargy, exercise intolerance and weight gain without significant increase in appetite. Cardiac abnormalities may develop with severe hypothyroidism including bradycardia and reversible cardiomyopathy. Neuromuscular dysfunctions include weakness of facial muscles. Alterations in skin and coat are common with dryness, excessive shedding, retarded regrowth of hair after cutting or trimming, and unilateral or bilateral non-pruritic alopecia that can be localised or diffused. In moderate to severe cases, thickening of skin occurs and myxoedema develops on the fore head and face, which is characterised by a puffy appearance and thickened folds above the eyes giving a sad or tragic look. Immunosuppression may lead to secondary pyoderma. Difficulty to maintain body temperature may lead to hypothermia; the classical hypothyroid dog is, therefore, heat seeker. Reproductive disorders may also develop including abnormal oestrous cycle, infertility, abortion and poor litter size in females and lack of libido, hypospermia and testicular atrophy in males.

In congenital or juvenile-onset hypothyroidism, disproportionate dwarfism and impaired mental development (cretinism) is observed in addition to well-recognised signs of adult-onset hypothyroidism. In primary congenital hypothyroidism, enlargement of the thyroid gland (goitre) may also be detected depending on the cause of hypothyroidism. In cats, lethargy and obesity are the most common clinical manifestations of hypothyroidism.

Excess of Thyroid Hormones / Hyperthyroidism

Excessive secretion of thyroid hormones resulting in clinical hyperthyroidism is most common in adult cats, but also develops rarely in dogs. Neoplasia of the thyroid gland, whether benign or malignant, is the most common cause of hyperthyroidism. Follicular cell adenomas, often developing in a thyroid with multinodular hyperplasia, are encountered more commonly than malignant thyroid tumours. The neoplastic cells release both T_4 and T_3 at an uncontrolled rate resulting in markedly elevated blood levels of both hormones. The plasma TSH concentration is mostly less than normal because excess hormones from thyroid depress the production of TSH by the pituitary gland.

The clinical signs of hyperthyroidism reflect abnormalities in several body systems mainly as a result of increased metabolic rate. Most effects of hyperthyroidism are obvious from the preceding discussion of the various physiological effects of thyroid hormones. The most common signs include weight loss in spite of a normal or increased appetite. Polydipsia and polyuria

occur in some cats. As skin protein synthesis and blood flow increase, the hair coat changes. Gastrointestinal signs are common and may include vomiting, diarrhoea and increased volume of faeces. Cardiovascular signs include tachycardia, systolic murmurs, dyspnoea, cardiomegaly and congestive heart failure. Thyroid hormones may also directly stimulate the central nervous system causing behavioural changes like nervousness, hyperkinesis and agitation in cats. Heat and stress intolerance, panting and respiratory distress may be related to decreased vital capacity, decreased pulmonary compliance, slightly elevated body temperature and cardiac stimulation.

Goitre / Hyperplasia of thyroid follicular cells

Goitre is a non-neoplastic and non-inflammatory enlargement of thyroid gland that may develop in all domestic mammals, man and birds. There are two general types of goitre – non-toxic goitre and toxic goitre. The non-toxic goitre produces either normal amount of thyroid hormones (simple goitre) or below-normal amounts of hormones (hypothyroid goitre), whereas toxic goitre produces excess amounts of hormones.

Simple goitre is a compensatory increase in thyroid glandular mass (hyperplasia and hypertrophy) so that the gland maximises iodine uptake and is able to synthesise and release normal amount of thyroid hormones. At this time, the patient is physiologically normal, but the gland is quite large. Hypothyroid goitre may occur because of excessive and prolonged deficiency of iodine, intake of goitrogenic compounds those interfere with thyroxineogenesis, and genetic enzymes defect in biosynthesis of thyroid hormones. All these factors lead to inadequate T_4 and T_3 synthesis and their decreased levels in blood. This is detected by the hypothalamus or pituitary to cause increase secretion of TSH, which results in hypertrophy and hyperplasia of follicular cells in the thyroid gland.

Toxic goitre is the term used for hyperthyroidism. In this type, the thyroid gland is increased to two to three times the normal size with tremendous hyperplasia and infolding of follicular cells lining into the follicles. The number of cells is increased several times and each cell in turn increases its hormonal secretion several fold. The changes in the thyroid gland are similar to those caused by excessive TSH.

TREATMENT OF THYROID HORMONE DISORDERS

A large number of compounds are available which directly or indirectly interfere with the synthesis, release or action of thyroid hormones. Some drugs are used only to suppress the clinical signs associated with thyroid hormone disorders. The treatment of thyroid hormone disorder varies depending on the type of thyroid disorder, degree of thyroid disorder, patient's age and some individual factors. With correct diagnosis and selection of suitable drug, low levels or high levels of thyroid hormone can be returned to normal levels in the body.

Classification

Drugs used in thyroid disorders are broadly classified into two groups: drugs used in deficiency of thyroid hormones and drugs used in excess of thyroid hormones.

I. Drugs used in deficiency of thyroid hormones
   1. Thyroid hormones and analogues
      e.g. levothyroxine, liothyronine, liotrix, tiratricol, thyroid extract and thyroglobulin.
   2. Miscellaneous drugs
      e.g. iodine.

II. Drugs used in excess of thyroid hormones
   1. Drugs inhibiting thyroid hormone synthesis/ Inhibitors of thyroid peroxidase
      e.g. thiamazole, carbimazole, propylthiouracil, methylthiouracil and benzylthiouracil.
   2. Drugs destroying thyroid tissue
      e.g. radioactive iodine (^{131}I, ^{123}I, ^{125}I).
   3. Drugs inhibiting thyroid hormone release
      e.g. iodides of sodium and potassium, organic iodides and iodine.
   4. Drugs inhibiting iodide trapping/Ionic inhibitors
      e.g. thiocynates, perchlorates, pertechnetate, nitrates and fluoborate.
   5. Miscellaneous drugs
   a. Antiadrenergic drugs
      e.g. propranolol and guanethidine.
   b. Other drugs
      e.g. ipodate, dibromotyrosine and diiodotyrosine.

In addition to above mentioned clinically useful drugs, several other drugs and compounds affect thyroid gland. Name and mode(s) of action of some anti-thyroid drugs is given in Table 50.1.

I. Drugs Used in Deficiency of Thyroid Hormones

1. Thyroid Hormones and Analogues

Thyroid hormones and analogues are used in the treatment of hypothyroidism regardless of the cause. Thyroid hormones can be supplemented as crude animal origin extracts or as synthetic preparations.

Levothyroxine-sodium / Thyroxine sodium

Levothyroxine sodium, also known as L-thyroxine, synthetic T_4, or 3,5,3',5'-tetraiodo-L-thyronine, is the sodium salt of laevo isomer of thyroxine, which is prepared synthetically for commercial use. Levothyroxine has the same effect as thyroid extracts, but is twice as active as the racemic (dl) form and is about 10-40 times more potent than d-isomer. An amount of 0.1 mg of levothyroxine is approximately equivalent to 65 mg (1 grain) of desiccated thyroid. Levothyroxine is available commercially in tablets and as a lyophilised powder for injection. It is considered as the drug of choice for most hypothyroid patients because thyroxine is the main secretory product of thyroid glands.

Pharmacological effects

The mode of action and pharmacological effects of levothyroxine-sodium is essentially similar to those observed with endogenous thyroxine secreted by the thyroid gland. In the target tissues, levothyroxine is mainly converted to T_3 and affects the rate of many physiological processes including fat, protein and carbohydrate metabolism. The increased oxidation rate, which underlies the calorigenic effect of thyroid hormones, is brought about by an effect on mitochondrial functions. Thyroid hormones also increase the oxygen consumption, blood volume, enzyme system activity, and growth and maturity.

Pharmacokinetics

The pharmacokinetics of levothyroxine varies from animal to animal and depends on drug preparations and several other factors. About 50% of the levothyroxine is absorbed after oral administration mainly from the ileum and colon and is influenced by intraluminal contents. In dogs, peak plasma concentration after oral administration occurs after 4-12 hours and serum half-life is approximately 12-15 hours. The t½ depends mainly on strong and extensive binding principally to thyroxine binding globulin and also on dose. Although the steady state concentration of T_4 is achieved at about 75 hours, the effects are not apparent for several days; maximum effect from single doses may not be achieved for as long as 10 days. Part of the absorbed dose of levothyroxine is deiodinated in peripheral tissue to the more active triiodothyronine (T_3) and rest is metabolised to inactive metabolites as discussed in the metabolism and excretion of thyroid hormones. Twenty to forty percent of the administered T_4 is excreted in faeces.

Side effects/Adverse effects

When administered at an appropriate dose to patients requiring thyroid hormone replacement therapy, levothyroxine does not produce significant side/adverse effects. Chronic overdosage may produce symptoms of hyperthyroidism, including tachycardia, polyuria, polydipsia, nervousness, panting, weight loss, diarrhoea and increase in appetite. Acute overdosage can produce thyrotoxicosis producing signs resembling the thyroid storm. The effects of overdosing usually appear 6 hours to 11 days after levothyroxine ingestion.

Treatment: Treatment of thyrotoxicosis is mainly supportive and symptomatic. Oxygen, artificial ventilation, cardiac glycosides, beta-adrenoceptor blockers, antipyretics and fluids have been suggested. Mild intoxication may subside with temporarily withdrawing the drug or by reduction of dosage.

Contraindications and precautions

Levothyroxine sodium is contraindicated in untreated adrenal insufficiency and in patients with acute myocardial infarction. It should be used with care in patients with concurrent hypoadrenocorticis, cardiac disease, diabetes mellitus, hypertension and in geriatric patients. Levothyroxine injection should not be mixed with other drugs or IV fluids. Because of different plasma protein binding activities of T_4 in humans and dogs, human dosage should not be used in dogs. Dosing must be carefully controlled to achieve TSH levels within the normal reference range.

Drug interactions

Levothyroxine increases actions of epinephrine and other sympathomimetics. Phenobarbital and other drugs which induce microsomal enzymes may increase the metabolism of thyroid hormones and may cause decrease in T_3 and/or T_4 levels. Thyroid hormones increase the catabolism of vitamin K dependent clotting factors and may increase anticoagulant effect of warfarin. Oestrogens may increase thyroid requirements by increasing thyroxine-binding globulin. Other drugs which compete for albumin binding sites increase T_4 levels, leading to decreased plasma half-life. Supplements of thyroid hormones in diabetic patients may alter the requirements of insulin.

Clinical uses

Levothyroxine sodium is commercially used for the treatment of hypothyroidism in all species. Although T_3 is the physiologically active thyroid hormone, T_4 is preferred product for supplementation because of its consistent potency and prolonged duration of action. Further, some tissues like central nervous system in particular have a great need for T_4 that is subsequently converted intracellularly to T_3. Levothyroxine therapy should be continued for long periods because the onset of clinical response to therapy usually takes 4 to 6 weeks and resolution of all clinical signs of hypothyroidism may take several months. As disposition of T_4 varies greatly, baseline T_3 and T_4 concentrations should be used to guide therapy. Animals should be watched periodically for side/adverse effects until a satisfactory maintenance dose is established. Levothyroxine is preferably taken on an empty stomach approximately half an hour before meals.

Dose

For hypothyroidism

Dogs : 0.02 – 0.04 mg/kg, PO, once daily or in 2 divided doses. Dose should be adjusted for each individual animal after about 8 weeks of therapy.

Cats : 0.02 mg/kg, PO, daily in divided doses.

Liothyronine

Liothyronine sodium (L-triiodothyronine sodium, T_3 thyronine sodium, T_3) is the sodium salt of synthetic triiodothyronine (T_3). It occurs as an odourless, light tan crystalline powder, which is very slightly soluble in water and slightly soluble in alcohol. Each 0.025 mg of liothyronine is equivalent to 65 mg (1 grain) of desiccated thyroid and about 0.1 mg of levothyroxine. Liothyronine therapy is indicated only when levothyroxine therapy has failed to achieve a response in dogs with confirmed hypothyroidism.

Pharmacological effects

Liothyronine has a similar action to levothyroxine, but is about 3 to 5 times as active as levothyroxine. Supplementation of T_3 alone usually corrects the serum T_3 levels, but not the abnormal serum T_4 concentration that prevents some tissues (e.g. CNS) from utilising it. In comparison to levothyroxine (T_4), liothyronine has a faster onset of action as well as a shorter biological half-life, which may be due to less plasma protein binding to thyroxine-binding globulin.

Pharmacokinetics

In contrast to levothyroxine, liothyronine is nearly completely absorbed by dogs after oral administration and also absorption is less affected by stomach contents. The peak plasma levels of liothyronine occur in 2 to 5 hours after oral administration and half-life varies between 5 and 6 hours in dogs. Although liothyronine has a similar action to levothyroxine, but it has a rapid onset of action, is more rapidly metabolised and has a shorter duration of action. The short half-life of liothyronine makes it difficult to avoid fluctuations during daily dosing.

Side effects/Adverse effects

Side/Adverse effects, contraindications and drug interactions of liothyronine are similar to those of levothyroxine.

Clinical uses

Although triiodothyronine (T_3) is the active intracellular hormone, the liothyronine is indicated only when levothyroxine therapy fails to show response in hypothyroidism. This is mainly due to its shorter duration of action, more frequent dosing, high cost and requirement for T_4 by some organs (e.g. CNS). Therefore liothyronine supplementation should be reserved for patients not responding to T_4 therapy i.e. those poorly convert T_4 to T_3 and

those poorly absorb T_4 after oral administration. Liothyronine is also used occasionally when a quicker onset of action is desired like in the treatment of myxoedema coma in humans and for preparing a patient for ^{131}I therapy for treatment of thyroid cancer.

Liothyronine is occasionally used in the T_3 suppression test for the diagnosis of mild hyperthyroidism in cats. Normally, T_3 has an inhibitory effect on the secretion of TSH (due to negative feedback mechanism) and injection of T_3 is followed by a fall in serum T4 levels. However in hyperthyroid cats, because of their autonomous production of T_4, the administered T_3 will have no suppressing effect on the high levels of serum T_4.

Dose

For hypothyroidism

Dogs : 0.004 – 0.006 mg/kg, PO, 3 times daily. Dose should be adjusted for individual animal.

Cats : 0.004 mg/kg, PO, 2 to 3 times daily.

T_3 suppression test

Cats : 0.025 mg (total), PO, every 8 hours for a total of 7 doses. Blood should be collected before first dose and after 4 hours of last dose. In hyperthyroid cat, there is little or no fall in serum T_4 levels following T_3 therapy, in contrast to greater than 50% suppression in normal cats.

Liotrix

Liotrix is a 4 : 1 mixture of levothyroxine sodium and liothyronine sodium. This combination product is formulated on the basis of proportion of both thyroid hormones secreted in the normal human patient. Although the physiological ratio is not the same in animals, the product can be occasionally used in veterinary medicine. Adverse effects are mainly due to overdose. Liotrix comes as a tablet for oral administration.

Dose

Dogs : 0.022 mg/kg, PO, 2 times daily.

Tiratricol

Tiratricol, also called TRIAC or 3,5,3-triodothyroacetic acid, is a thyroid hormone analogue. It is indicated in the management of thyroid hormone resistance syndrome and is used in combination with levothyroxine to suppress thyroid-stimulating hormone production in patients with thyroid cancer.

Thyroid extract

Thyroid extract (Thyroid BP/USP) is a dried thyroid extract obtained from defatted thyroid glands of cattle, sheep and pigs. It is standardised by chemical means and contains 0.17 % to 0.23% of iodine by weight in organic combination with thyroid tissue. It is available in tablet forms and is relatively cheap source of thyroactive principles for oral administration. Thyroid extract is unstable on storage and stored samples have shown to vary considerably in their thyroxine content. Further tolerance to thyroid extract develops after some time due to development of antibodies. Therefore, adjustment of dosage may be necessary.

Thyroid extract is less used presently, because of its unreliable efficacy and availability of synthetic hormones.

Dose

All species : 1 – 2 mg/kg, PO, 1 to 2 times daily.

Thyroglobulin

Thyroglobulin (Proloid) is a purified extract of frozen hog thyroid gland. It is assayed both chemically and biologically. Its activity is similar to thyroid extract but has no advantage over it. It is seldom used in therapy.

2. Miscellaneous Drugs

Iodine

Iodine is an essential trace element, required by the thyroid hormones thyroxine and triiodotyronine. As a minute amount of iodine intake is necessary for the normal synthesis of thyroid hormones, iodine may be provided to animals in iodine-deficient regions. Mineral supplements and salt licks containing about 0.03% to 0.06% of potassium iodide or the more stable iodate helps in prevention of iodine deficiency related manifestations. Sometimes sodium or potassium iodide (1 mg/kg) is used by oral route for the treatment of iodine-deficient simple goitre. In human beings, addition of iodine to table salt helps in prevention of mental retardation, a generalized disorder seen in babies or small children when they are rendered hypothyroidic by lack of the iodine.

II. Drugs Used in Excess of Thyroid Hormones/ Hyperthyroidism

Hyperthyroidism is a common problem and may be treated surgically or pharmacologically. Surgery is preferred only when there are mechanical problems due to compression of the trachea. Drugs used to control hypothyroidism primarily lower the functional capacity of the hyperactive thyroid gland. These drugs are used in the pre-operative preparation of hyperthyroid patients for thyroidectomy or for long-term management of hyperthyroidism. Treatment with pharmacological agents often control hyperthyroidism, but in many cases disease in not cured completely because drugs do not alter the underlying autoimmune mechanisms or do not remove the primary cause of disease. Some drugs are used only to suppress the clinical signs of hyperthyroidism.

1. Drugs Inhibiting Thyroid Hormone Synthesis/ Inhibitors of Thyroid Peroxidase Enzyme

Several compounds related to thiourea have a specific depressant effect on thyroid activity and thyroxine production. Of these, compounds containing a thioureylene group constitute one of the most important groups of antithyroid agents. These drugs are commonly called antithyroid drugs, thionamides or thioureylenes and are effective in both human beings and animals. They inhibit synthesis of thyroid hormones mainly by blocking the action of thyroid peroxidase enzyme.

Thiamazole

Thiamazole, also known as methimazole or tapazole, is a sulphur containing imidazole-derivative antithyroid drug. It occurs as a white to pale crystalline powder, which is freely soluble in water and alcohol. Presence of the thiocarbamide group (S-C-N) is essential for its antithyroid action.

Mechanism of action

Thiamazole primarily inhibits formation of thyroid hormones by interfering with the incorporation of iodide into tyrosyl residues of thyroglobulin and also coupling of iodotyrosine residues to form T_4 and T_3. This occurs due to inhibition and inactivation of the peroxidase enzyme and subsequent interference with oxidation of iodide ion and iodotyrosyl residues.

Over a period of time, the inhibition of hormone synthesis results in depletion of stores of iodinated thyroglobulin and thyroid hormones. Antithyroid drugs do not affect release of T_3 and T_4; therefore, their effects are not apparent till thyroid is depleted of its hormone contents. It is suggested that the coupling reactions are more sensitive to thiamazole and other antithyroid hormones than the iodination reactions.

Pharmacological effects

Thiamazole decreases the output of thyroid hormones from the gland and cause a gradual reduction in signs and symptoms of hyperthyroidism. The basal metabolic rate and pulse rate returns to normal over a period of 3-4 weeks. As thiamazole alleviates only the symptoms of hyperthyroidism, clinical signs usually recur when the drug is discontinued.

Pharmacokinetics

After oral administration, thiamazole is absorbed and distributed throughout the body water. However, its bioavailability and elimination half-life are variable in animals. In normal cats, the bioavailability is reported to be 45-98%, whereas the plasma elimination half-life varies from 2.5-10 hours. Because of the high plasma protein-binding activity of thyroid hormones and availability of pre-formed thyroid hormones in the gland, the plasma levels of T_3 and T_4 decline slowly. Significant reduction in the levels of the thyroid hormones is usually reported within 1-3 week of starting the drug.

Side effects/Adverse effects

Thiamazole is reported to produce vomiting, anorexia, lethargy, pruritus, thrombocytopenia, hepatotoxicity, lymphocytosis, eosinophilia and transient leucopenia. Self-induced excoriations of face and neck may occur within the first 6 weeks of therapy. The GI adverse effects generally develop within the first month of treatment and usually resolve with time despite continuation of therapy. Occasionally, cats receiving thiamazole may exhibit prolonged bleeding despite normal platelet counts. High doses may produce severe hepatopathy and blood dyscrasias, in particular severe thrombocytopenia and agranulocytosis. High levels of thiamazole cross the placenta and may induce hypothyroidism in kittens born to queens receiving the drug.

Treatment: Thiamazole has no specific antidote. Thiamazole intoxication may be treated symptomatically and supportively.

Contraindications and precautions

Thiamazole is contraindicated in patients those are hypersensitive to it. It should be used cautiously in patients with a history of or current haematological disorders, hepatopathies or autoimmune diseases. Thiamazole should be used very cautiously in pregnant animals. Because many of the side effects of thiamazole are dose-dependent, the daily dose should be maintained at lowest effective. To maintain normal levels of thyroid hormones and to monitor for adverse reactions during the first 3 months of treatment (when the most serious side effects develop), complete blood count and serum thyroid hormones determination should be repeated at 2 to 4 weeks intervals.

Drug interactions

No significant drug interactions have been reported for thiamazole. However, it may show interaction when taken along with oral anticoagulants (e.g. warfarin), beta adrenoceptor blockers (e.g. propranolol), anti-diabetes drugs, digitalis (e.g. digoxin), methylxanthines (e.g. theophylline) and vitamins.

Clinical uses

Thiamazole is considered as drug of choice for the treatment of hyperthyroidism in cats. Thiamazole is effective, longer acting and safer than propylthiouracil, the main antithyroid drug used in human medicine. Thiamazole is useful both for long-term management of hyperthyroidism and in the pre-operative preparation of hyperthyroid patient for surgery. It is useful before surgery to decrease T_4 concentrations to a safe and acceptable level (usually 2 to 4 weeks).

Dose

Cats : 2.5 – 10 mg (total), PO, in 2 to 3 divided doses. Serum T_4 levels should be monitored carefully at 3 weeks for response. If the T_4 levels fall to low or low normal, dose may be decreased by 2.5-5 mg increments; if concentrations fail to fall, dose may be increased in 5 mg increments.

Carbimazole

Carbimazole is a carbethoxy derivative of thiamazole. Carbimazole is considered a pro-drug as its antithyroid action is due to its conversion to thiamazole after absorption. The active metabolite thiamazole prevents the thyroid peroxidase enzyme from coupling and iodinating the tyrosine residues on thyroglobulin, hence reducing the production of the thyroid hormones T3 and T4. Carbimazole appears to be equal in efficacy and safer than thiamazole. Carbimazole is currently used in Europe and some other countries and is considered as drug of choice for hyperthyroidism. The pharmacology and clinical uses of carbimazole are similar to those of thiamazole.

Dose

Cats & dogs : 10 – 15 mg (total), PO, daily in divided dose for 1 to 3 weeks. The dose of individual animal should be adjusted to the lowest effective level.

Propylthiouracil

Propylthiouracil (PTU) or 6-n-Propylthiouracil (PROP) is a thioamide drug used to treat hyperthyroidism. It is used widely in human medicine but has a limited importance in animals. Similar to thiamazole, PTU inhibits the enzyme thyroperoxidase which normally acts in thyroid hormone synthesis. In this regard, it is about 10 times less potent than thiamazole. In addition to blocking hormone synthesis, propylthiouracil inhibits the peripheral deiodination of thyroxine to triiodothyronine. Although it effectively reduces circulating thyroid hormones to normal levels, the incidence of adverse reactions in cats is much higher (20-25%). Important side effects of propylthiouracil include vomiting, anorexia, lethargy, thrombocytopenia, bleeding disorders, immune-mediated haemolytic anaemia and lupus-like syndrome. Serum antinuclear antibodies may develop particularly at high doses.

Dose

Cats : 50 mg (total), PO, 3 times daily. Dosage may be adjusted as described for thiamazole and carbimazole.

Other drugs

Two other less commonly used anti-thyroid drugs are methylthiouracil and benzylthiouracil. Both drugs belong to the thiouracil group and mediate effects through inhibition of thyroid

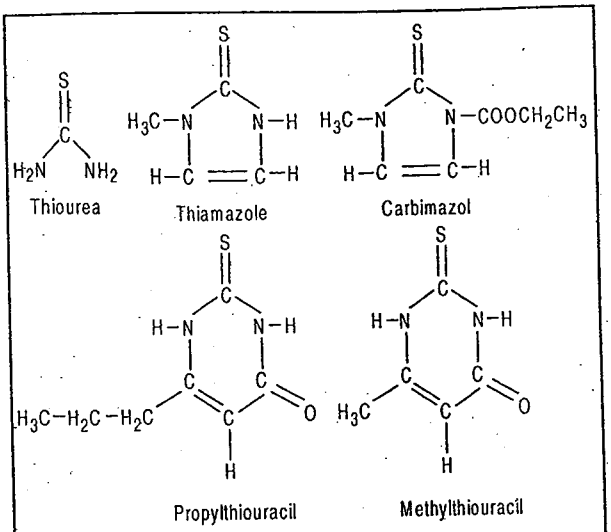


Fig. 50.5. Chemical structures of selected antithyroid drugs of thionamide type.

peroxidase enzyme. Methylthiouracil is equal in potency to propylthiouracil, but is rarely used nowadays as allergic manifestations and other adverse effects are more frequently observed with its use. Benzylthiouracil has been associated with severe adverse effects in humans, notably vasculitis and glomerulonephritis, as well as isolated reports of lung damage.

II. Drugs Destroying Thyroid Tissue

Radioactive iodine

Several radioactive isotopes of iodine viz. ^{123}I, ^{125}I and ^{131}I are available and are used for different purposes. Among these, the radioactive ^{131}I is most commonly used to destroy thyroid tissue because it emits β-particles as well as γ-rays and has a physical half-life of 8 days. The ^{123}I, mainly a γ-emitter, has a short physical life of 13 hours that permits a relatively brief exposure of radiation during thyroid scan. The long half-life of about 60 days makes ^{125}I unsuitable of therapeutic purposes. The radioactive iodine therapy is the treatment of choice if cure is desired in cats with intrathoracic thyroid tumours or metabolic carcinoma but for which surgery is undesirable. Radioactive iodine is used as the sodium salt of ^{131}I.

Mechanism of action

The chemical behaviour of radioactive iodine (^{131}I) is similar to stable isotope of iodine (^{125}I). Therefore, radioactive iodine is not distinguished from the ordinary iodine by the thyroid gland and is actively taken up and stored in the thyroid gland. The ^{131}I is concentrated by thyroid gland and is incorporated in the colloid, where it emits radiation from within the follicles. The radioactive emissions, in particular β-particles, destroy the functional and regeneration capacity of the thyroid cells. The follicular cells get necrosed and the colloid disappears, followed by fibrosis. The ^{131}I is preferred because it emits β-particles, which penetrate only 0.5 – 2 mm of tissues, thus limiting damage to neighbouring tissue including parathyroid glands and other cervical structures (the γ-rays emitted by ^{131}I pass through these tissues). In the radioactive therapy, normal tissue is relatively spared from destruction by the unstable ion because normal tissue is quiescent compared with hyperactive tissue.

Pharmacological effects

The pharmacological effect of radioactive ^{131}I is observed only on the thyroid gland and is dose-dependent. When small doses of ^{131}I are used, thyroid function is not disturbed much as some of follicles, usually in the periphery of gland, retain this function. In a suitable therapeutic dose(s), large amounts of radioactive iodine gains access to the gland and characteristic cytotoxic actions of ionising radiations are observed. Beginning a few weeks after treatment, the symptoms of hyperthyroidism gradually abate and clinical signs of euthyroidism generally occur. The first sign generally observed is normalisation of the appetite and weight gain. Therefore with properly selected doses of ^{131}I, it is possible to destroy the thyroid gland completely without detectable injury to adjacent tissues.

Pharmacokinetics

Sodium ^{131}I is administered IV, SC, or orally. After administration, ^{131}I is rapidly incorporated into the thyroid follicles. The ^{131}I has a short half-life of 8 days. After 2 months of therapy, the radioactivity effectively disappears from the body.

Side effects/Adverse effects

Side/Adverse effects of radioactive iodine treatment are rare. Pain or discomfort in the area of thyroid gland presumably reflects radioactive thyroiditis and usually resolve within several days of therapy. Hypothyroidism may develop in some cats (<5%) treated with radioactive iodine therapy and may temporarily require replacement therapy.

Contraindications and precautions

Radioactive iodine therapy is contraindicated during pregnancy and lactation because destruction of foetal thyroid may occur resulting in cretinism and other abnormalities. Radioactive iodine should not be used in larger toxic nodular goitre and in severe thyrotoxic cardiovascular disease. In humans, radioiodine is not used in young patients because they are more likely to develop hypothyroidism later and would require life-long T_4 treatment. Radioactive iodine treated cats continue to excrete a small amount of radiation for 2 to 4 week; therefore, a close contact with the cat should be minimised during this time. Isolation facilities are essentially required when radioactive iodine is used in animals. All personnel handling the cats, cages, food dishes and excreta should wear long laboratory coats, disposable plastic gloves and film badges. All material removed from the cage must be handled as radioactive waste and disposed off accordingly.

Clinical uses

Radioactive iodine (^{131}I) has been successfully used in the management of feline hyperthyroidism and it provides a simple and safe treatment. Indications for ^{131}I include, intolerance to drug treatment, recurrence following surgery, or when surgery is contraindicated due to location of the tumour or condition of the patient. The success rate with radioactive iodine therapy for treatment of feline hyperthyroidism is as much as 90%. Up to 10% of treated cats, however, require as second treatment with radioactive iodide. Presence of clinical signs consistent with hyperthyroidism 3 months after treatment with radioactive iodine may indicate the need for re-treatment. The major disadvantages of iodine therapy include limited accessibility (most practitioners do not have access to radioactive iodine), high cost and requirement for stay in hospital till radioactivity declines significantly in the patient.

Radioactive uptake studies are also utilised occasionally in the diagnosis of thyroid disorders. Measurement of thyroidal accumulation of a tracer dose is useful in the diagnosis of hyperthyroidism, hypothyroidism and goitre, and also to determine the response of thyroid to TSH (a failing thyroid that responds to TSH by increased uptake of ^{131}I indicates pituitary TSH deficiency/secondary hypothyroidism, while a failing thyroid that does not respond to TSH indicates primary thyroid dysfunction). Radioactive uptake studies may also be used for localisation of metastatic deposits of thyroid cancer in the lungs and bones.

Dose

Dose of ^{131}I is calculated on the basis of previous tracer studies, severity of clinical signs and size of thyroid gland. In practice, dose between 2 to 6 mci of ^{131}I is used in cats by PO, SC or IV route. It produces euthyroidism in majority of cases. Rarely, a high dose of 10 to 30 mci is used to destroy adenomatous thyroid tissues as well as the normal thyroid tissue.

III. Drugs Inhibiting Hormone Release

Iodine and Iodide

Iodine / Iodine in the form of lugol's iodine or potassium iodide is the oldest agent used in the management of thyroid disorders. As mentioned earlier, iodide is required for the synthesis of thyroid hormones and is used in the treatment of iodine-deficiency goitre. Paradoxically, iodide in high doses is used for the control of signs and symptoms of hyperthyroidism. The response of iodine or iodide is identical because iodine is reduced in the intestine to iodide.

Mechanism of action

The exact mechanism of action of iodine in hyperthyroidism in ill understood. High iodide content in blood appears to influence almost all important aspects of iodine metabolism by the thyroid gland. High concentrations of iodide limit its own transport and inhibit the iodination of tyrosine, thus decreasing the supply of stored thyroglobulin. High iodide content in blood inhibits thyroid hormone release, which is clinically very important. This action is rapid and efficacious in severe thyrotoxicosis. Although the exact mode is not yet completely understood, iodide probably antagonises the ability of both TSH and cAMP to stimulate the proteolysis and hormone release. Iodide also reduces vascularity of the gland, which becomes smaller and firmer. The thyroid status starts returning to normal at a rate commensurate with complete stoppage of hormone release from the gland.

Pharmacological effects

The effects of iodide in patients with hyperthyroidism are often drastic and rapid. The beneficial effects are usually seen within 24 hours and the basal metabolic rate may fall at a rate comparable to that following thyroidectomy. The maximal effect in attained after about 10 to 15 days of continuous iodide therapy, when the signs and symptoms of hyperthyroidism may have greatly improved. During this period, there is reduction in the vascularity and swelling of the gland. The cells become smaller, and colloid reaccumulates in the follicles. However after a few weeks of daily therapy, the beneficial effect disappears, thyroid ceases to respond to iodide, and hyperthyroidism may return to its initial intensity or may become even worse. This is diagnosed as the 'thyroid escape phenomenon'.

Pharmacokinetics

The pharmacokinetics literature on iodides in domestic animals is scarce. After oral administration, iodine is reduced in the intestine to iodide, which is then absorbed rapidly. After absorption, iodide accumulates in thyroid and function as the fast acting thyroid inhibitor. The effect is usually discernible within 24 hours.

Side effects/Adverse effects

Side/Adverse effects of iodine/iodide are minor and may include hypersensitivity, anorexia, vomiting, soar mouth and throat, rashes and ulceration of mucous membranes. Excessive intake of iodine in animals can cause excessive tearing, nasal discharge, scaly hair coat/dandruff, decreased milk production and diarrhoea. Occasionally, marked sensitivity to iodide or organic preparations which contain iodine may be seen when they are administered IV. Thrombotic thrombocytopenic purpura and fatal periarteritis nodosa attributed to hypersensitivity to iodide have been described in human beings.

Treatment: The symptoms of iodism (chronic overdosage) disappear spontaneously within a few days after stopping the administration of iodide. The renal excretion of iodide in severe iodism may be enhanced by procedures which promote chloride excretion (e.g. osmotic diuretics and salt loading). If thyrotoxicosis crisis occurs, it is treated by simultaneous administration of propranolol and IV fluids.

Contraindications and precautions

Iodides should not be given to lactating or pregnant animals because excess iodide is reported to cause abortion in animals. Iodide should not be used for long-term treatment of hyperthyroidism because its antithyroid action tends to diminish and patients may not achieve the euthyroid state.

Clinical uses

Iodine and iodide are used before thyroidectomy to block the release of thyroxine and triiodothyronine and to reduce the vascularity of thyroid gland. They can also be used in conjunction with antithyroid drugs and propranolol in the treatment of thyrotoxic crisis. Prior to surgery, iodide is sometimes employed alone but more frequently is used after an antithyroid drug has controlled hyperthyroidism.

The dosage or form in which iodide is administered bears little relationship to the response achieved in hyperthyroidism, provided not less than the minimal effective amount is given. Strong iodine solution (Lugol's solution or aqueous iodine oral solution) consisting of 5% iodine and 10% potassium iodide and yielding a dose of 6.3 mg of iodine per drop is widely used. Saturated solution of potassium iodide also is available containing 38 mg per drop.

Dose

Lugol's solution or aqueous iodine oral solution

Cats : 3 – 5 drops, PO, daily for 7 to 14 days before surgery.

Saturated solution of potassium iodide

Cats : 1 – 2 drops, PO, daily in gelatine capsule beginning 10 days before surgery.

IV. Drugs Inhibiting Iodide Trapping / Ionic Inhibitors

Ionic inhibitors are substances which interfere with the accumulation/concentration of iodide by the thyroid gland. These

agents are mainly inorganic monovalent anions of a size similar to that of iodide. Because of their similar hydrated ionic size, they compete with iodide for the sodium-iodide symporter in the membrane of follicle cells and inhibit iodide trapping by the thyroid. These agents also cause loss of inorganic iodide, which has already been taken up. However, these agents have limited application in therapeutics because of their adverse and toxic effects.

Thiocyanate

Thiocyanate (SCN^-) is the conjugate base of thiocyanic acid. Common derivatives include the colourless salts potassium thiocyanate and sodium thiocyanate. Thiocyanate is produced also following the enzymatic hydrolysis of certain plant glycosides, vegetables (e.g. cabbage) and drugs (e.g. nitroprusside). Thiocyanate has affinity for iodide transport system (sodium-iodide symporter) almost similar to that of iodide ion. However, it is not concentrated by the thyroid gland. In large amounts, thiocyanate also inhibits the organification of iodine. Accumulation of thiocyanate in body causes liver, kidney, bone marrow and brain toxicity. Dietary precursors of thiocyanate may be a contributing factor in endemic goitre in human beings in certain parts of the world, where the intake of iodine is low.

Perchlorate

Perchlorates (ClO_4^-) are the salts derived from perchloric acid ($HClO_4$). Some salts of perchlorate (e.g. potassium perchlorate) have been used as a drug for more than 50 years to treat thyroid gland disorders. Perchlorate effectively blocks iodide trapping and is about 10 times as active as thiocyanate. Perchlorate affects only the thyroid gland and has no storage or effect anywhere else in the body. Although perchlorate can be effectively used to control hyperthyroidism, it may cause serious adverse effects like agranulocytosis, fever, rashes and aplastic anaemia. The effects of perchlorate on the thyroid gland are generally considered reversible once exposure stops.

Other ions

Several other monovalent anions like fluoborate (BF_4^-), nitrate (NO_3^-) and pertechnetate (TcO_4^-) have also been found to be effective iodide inhibitors. Lithium also has antithyroid function, but its principal effect is decreased secretion of thyroxine and triiodothyronine.

V. Miscellaneous Drugs

Several groups of drugs other than those discussed above are useful also in hyperthyroidism. These drugs mainly control clinical signs of hyperthyroidism and are used as adjuncts to primary antithyroid drugs.

a. Antiadrenergic Drugs

Propranolol

Beta-adrenoceptor blockers like propranolol are very useful in decreasing many of the signs and symptoms of hyperthyroidism. Thyroid hormones appear to increase the number or sensitivity of beta-adrenoceptors in myocardium and frequently produce tachycardia, hypertrophic cardiomyopathy, congestive cardiomyopathy and cardiac arrhythmias in hyperthyroid cats. Beta-adrenoceptor blockade by propranolol can reduce these sympathetic over activity manifestations. In addition, propranolol reduces concentration of T_3 hormone by inhibiting conversion of T_4 to T_3 by peripheral tissues in patients with hyperthyroidism. However, beta-adrenoceptor blockers have little effect on the thyroid function and hypermetabolic state.

Propranolol is mainly used during thyrotoxicosis crisis (thyroid storm/severe hyperthyroidism) along with antithyroid drugs. It is also used along with iodide for pre-operative preparation before subtotal thyroidectomy.

Dose

Cats : 2.5 mg (total), PO, 2 times daily. Dose may be increased to 7.5 mg (total), 3 times daily to control clinical signs effectively, if required.

Table 50.1: Mode(s) of action of important antithyroid agents.

Process	Examples of Inhibitors
1. Iodide trapping	Complex anions: Perchlorate, thiocyanate, fluoborate and pertechnitate.
2. Iodination of thyroglobulin	Thionamides: Thiamazole, carbimazole, and propylthiouracil. Anions: Thiocyanate. Iodide (high concentration).
3. Coupling reactions	Thionamides: Thiamazole, carbimazole, and propylthiouracil.
4. Hormone release	Lithium salt. Iodide (high concentration).
5. Peripehral deiodination of T_4 to T_3	Thionamides: Propylthiouracil. Adrenoceptor blockers: Propranolol. Amiodarone.
6. Destruction of thyroid tissue	Radioactive iodine (^{131}I).
7. Hormone inactivation /excretion	Hepatic microsomal enzyme inducers: Phenobarbital and rifampicin.
8. Hormone action	Thyroxine analogues. Amiodarone.

Guanethidine

Guanethidine is an adrenergic neuron-blocking drug that is used occasionally in human patients to ameliorate the exophthalmos of hyperthyroidism (that is not relieved by antithyroid drugs). It is used in eye drops and acts by relaxing the sympathetic innervated smooth muscle that causes eyelid retraction.

b. Other Drugs

Ipodate

Ipodate sodium (Sodium iopodate) is an iodine-containing radiopaque contrast media used for X-rays. In emergency situations for thyroid storm, ipodate can be administered by oral route. After metabolism, it releases iodine into circulation, helping bring the T_3 and T_4 levels back to normal. Ipodate also inhibits the conversion of T_4 to T_3, the more active form of the thyroid hormone. Ipodate has significantly higher incidence of side/adverse effects and is not routinely used.

Other drugs

Dibromotyrosine and diiodotyrosine (DIT) are other antithyroid preparations, used occasionally in treatment of hyperthyroidism.

CALCITONIN

Calcitonin (CT) is a hormone produced and secreted by the paracellular C-cells of the thyroid gland. It reduces blood calcium ion concentration and produces an effect which is opposite to that of parathyroid hormone (PTH).

Synthesis and secretion

Calcitonin is a polypeptide with a molecular weight of about 3400 and a chain of 32 amino acids. It contains a disulphide bridge present between amino acids 1 and 7. The structure of calcitonin differs considerably between species. Like other peptide hormones, it is synthesised initially as pre-prohormone in the rough endoplasmic reticulum and then converted to a prohormone (procalcitonin). The procalcitonin is transported to the Golgi apparatus where it is converted to calcitonin prior to packaging in the secretory granules. The secretion of calcitonin occurs by exocytosis from granules. It is secreted continuously under normo-calcaemic states but its rate of secretion is increased with hypercalcaemia. The half-life time of calcitonin is 4-12 minutes, but its action lasts for several hours.

Regulation of secretion

Biosynthesis and secretion of calcitonin is determined mainly by the plasma calcium concentration. Rise in plasma Ca^{++} increases, while fall in plasma Ca^{++} decreases calcitonin release. Hyperplasia of C-cell occurs in response to long-term hypercalcaemia. Gastrin, cholecystokinin, secretin and glucagon all have been demonstrated to stimulate calcitonin release under experimental condition in animals. Calcitonin secretion is also stimulated by catecholamines, but they have minor role in the secretion of calcitonin under physiological conditions.

Mechanism of action

The hypocalcaemic and hypophosphataemic effects of calcitonin are caused predominantly by inhibition of osteoclastic bone resorption. It interacts directly with calcitonin receptors (CT-R), which are G protein-coupled receptor and works by activating the G-proteins G_s and G_q. The activation of CT receptors present on osteoclast produces a rapid and profound decrease in ruffled border surface area, thereby diminishing resorptive activity. Calcitonin also inhibits temporarily the parathormone-stimulated bone resorption. Calcitonin may inhibit the effect of parathormone on osteolysis, although it does not block activation of cell adenylate cyclase by PTH and does not inhibit PTH-induced uptake of Ca^{++} into bone. Calcitonin promotes renal excretion of Ca^{++} and phosphate probably by involvement of cAMP.

Physiological effects

The physiological actions of calcitonin are generally opposite to those of parathyroid hormone. Calcitonin decreases calcium and phosphate levels in the extracellular fluid.

Bone: The biological effects of calcitonin are most evident in bones. It acts on the bones and inhibits bone resorption by osteoclasts. It decreases transfer of both calcium and phosphate to the extracellular fluid and increases movement of phosphate from the extracellular fluid into bones. These effects of calcitonin result in development of varying degree of hypocalcaemia and hypophosphataemia, which are opposite to the effects of PTH. The action of calcitonin is not dependent on the vitamin D as it acts both in vitamin D deficiency and after supplementation of vitamin D.

Kidneys: Like parathyroid hormone, calcitonin decreases renal tubular reabsorption of phosphates to cause phosphaturia. In addition, it decreases renal tubular reabsorption of calcium, sodium and chloride. The physiological significance of calcitonin on renal electrolyte excretion in mammals, however, is minor.

Gastrointestinal tract: Calcitonin decreases GI activity directly by inhibiting gastric acid secretion and indirectly by inhibiting gastrin secretion. Calcitonin decreases calcium absorption from gut, but it plays minor role in the calcium homeostasis.

DISORDERS OF CALCITONIN

Clinical syndromes associated with abnormalities in secretion of calcitonin are recognised less frequently in both animals and man.

Deficiency of Calcitonin / Hypocalcitoninism

Specific disease syndrome resulting from a lack of calcitonin secretion has not been recognised in either man or animals. However in thyroidectomised animals, hypercalcaemia may develop due of lack of calcitonin synthesis and secretion. In women, excessive bone resorption and inadequate new bone formation during remodelling have been associated with post-menopausal osteoporosis.

Excess of Calcitonin / Hypercalcitoninism

Hypersecretion of calcitonin has been reported in human beings and bulls with thyroid C-cell neoplasms. The high calcium diets fed to bulls over a long period may be related to the pathogenesis of the neoplasm. Cows do not develop proliferative C-cell lesions under similar dietary conditions, probably because of high physiological requirement of calcium imposed by pregnancy and lactation. The C-cell thyroid carcinomas also occur in dogs and horses sporadically. Osteosclerotic changes have been reported in bulls with long term and excessive secretion of calcitonin secretion.

TREATMENT OF CALCITONIN DISORDERS

Calcitonin

Calcitonin is available commercially as calcitonin porcine (natural) and calcitonin salmon (initially obtained from salmon fish, but now prepared by either recombinant DNA technology or chemical peptide synthesis). Synthetic human calcitonin is now also available. Calcitonin salmon (salcatonin) is approximately 50 times more potent than calcitonin human on a per weight basis and is preferably used in both human and veterinary medicines. Potency of calcitonin is expressed in international units (I.U.).

Pharmacological effects

The mechanism of action and pharmacological effects of calcitonin porcine, calcitonin salmon or calcitonin human are similar to those of endogenous calcitonin released by C-cells of thyroid gland. Exogenous calcitonin acts on bone inhibiting osteoclastic bone resorption. It also reduces tubular reabsorption of calcium, phosphate, sodium, potassium and chloride and promotes their renal excretion.

Pharmacokinetics

Like other peptide hormones, calcitonin is inactive orally. After IV administration, calcitonin produces immediate effect. After

Table 50.2: Amino acid sequences of human, salmon and eel calcitonin.

Species	Amino acid sequence
Human	Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro.
Salmon	Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro.
Eel (Elcatonin)	Ser-Asn-Leu-Ser-Thr-Asu-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Val-Gly-Ala-Gly-Thr-Pro.

SC or IM injection, the pharmacological effect (decline in calcium concentration) starts within 15 minutes, peaks at about 4 hours and lasts for approximately 10 hours (8-14 hours). Calcitonin is metabolised rapidly in the liver, kidneys, blood and some peripheral tissues. The plasma half-life of calcitonin is about 10 minutes. Salmon calcitonin has a longer half-life probably due to resistance to metabolic degradation.

Side effects/Adverse effects

Side/Adverse effects of calcitonin are not well documented in animals. In dogs and humans, calcitonin has been reported to produce anorexia, vomiting and diarrhoea. Inflammatory reactions may occur at the site of injection. It may produce hypocalcaemia, but does not produce tetany. Porcine (natural) calcitonin may contain traces of thyroid hormones and can lead to production of antibodies and development of allergic reactions. Tachyphylaxis may occur in some dogs, probably as a result of compensatory increase in the secretion of parathyroid hormone.

Contraindications and precautions

Calcitonin is contraindicated in animals hypersensitive to it. Young animals are more sensitive (about 100 times) to calcitonin than are adult animals. Calcitonin should not be used in lactating animals as it has been shown to inhibit lactation.

Clinical uses

The clinical use of calcitonin in hypercalcaemic states is limited in both human and veterinary medicines due to its short duration of action and rapid development of resistance. In human beings, it is used occasionally to suppress the bone resorption of Paget's disease and in osteoporosis. It is also useful in the treatment of hyperparathyroidism and vitamin D poisoning. In veterinary medicine, calcitonin is used in small animals mainly as adjunctive therapy to reduce bone resorption and to control hypercalcaemia in poisoning due to rodenticides containing ergocalciferol (vitamin D_2)

Dose

For hypervitaminosis D (Toxicity)

Dogs & cats : 4 – 6 IU/kg, SC, 2 to 3 times daily.

: 4 IU/kg, IV (loading dose for severe toxicity) followed by 4 – 8 IU/kg, SC, 1 to 2 times daily.

PARATHYROID HORMONE

Parathyroid Gland

The parathyroid in most species consists of two pairs of glands, which secrete the parathyroid hormone (PTH) or parathormone. The parathyroid hormone along with calcitonin and vitamin D regulates calcium homeostasis in the body.

The parathyroid glands are usually located in the anterior cervical region and consist of four glands in most species. In some species, they are embedded in the thyroid gland but in others they are distinct entities. In dogs and cats, the parathyroid glands are situated close to the thyroid glands, whereas in cattle and sheep the two parathyroids are situated on dorsal and medial surface of thyroid and the other two are located a considerable distance cranial to the thyroid. Pigs have only a single pair of parathyroids found cranial to the thyroid gland.

The parathyroid glands of adult human being and many species contain mainly chief cells and a small to moderate number of oxyphil cells. The oxyphil cells are absent in some species (e.g. rat, chicken and lower animals) and in young human beings. The chief cells are believed to secrete most, if not all, of the parathyroid hormone. The function of the oxyphil cells is not certain; they are believed to be modified or depleted chief cells which do not secrete hormone.

Parathyroid Hormone

The parathyroid hormone (PTH), also called parathormone or parathyrin, is a large singe-chain polypeptide hormone synthesised and secreted by the chief cells of the parathyroid gland. It consists of 84 amino acids residue with a molecular weight of about 9500. The first 34 amino acid residues contain the hormonal activity of the molecule.

Synthesis, secretion, and metabolism

Synthesis and release: The synthesis of parathyroid hormone is similar to the synthesis of other protein hormones. It is synthesised initially as a component of pre-proparathormone of 115 amino acids in the rough endoplasmic reticulum, then cleaved by 25 amino acids to form a prothormone. A 6 amino acids pro portion is cleaved by the Golgi apparatus resulting in formation of 84 amino acid residues parathormone which is packaged in the secretory granules in cytoplasm of the cells. Parathyroid hormone is released by the process of exocytosis.

Metabolism: Parathyroid hormone is rapidly metabolised by the liver and kidneys and has a relatively short half-life of 5-10 minutes in blood. Metabolism of PTH releases fragments which circulate in blood. Fragments are also released by proteolysis of parathormone within the parathyroid gland.

Regulation of secretion

Parathyroid hormone release is regulated mainly by the Ca^{++} ion concentration in blood. There appears to be an inverse correlation between the plasma calcium concentration and the parathormone concentrations i.e. release of parathormone is stimulated by a fall in plasma Ca^{++} levels and inhibited by a rise in the ionised plasma Ca^{++} levels. Prolonged hypocalcaemia causes hypertrophy and hyperplasia of parathyroid glands, while sustained hypercalcaemia causes opposite effect. An increase of calcium ions in the extracellular fluids rapidly inhibits uptake of amino acids by chief cells, synthesis of PTH and secretion of

stored PTH. Thus Ca^{++} *per se* appears to regulate parathyroid gland growth as well as hormone synthesis and secretion.

The magnesium ion has an effect on parathyroid secretion similar to that of calcium, but its effect is not equipotent to that of calcium. Changes in phosphate concentrations in plasma affect PTH secretion indirectly by altering Ca^{++} concentrations. Epinephrine also stimulates PTH secretion through stimulation of beta-adrenoceptors. The active form of vitamin D calcitriol inhibits expression of PTH gene in the parathyroid gland cells. No effective control by nervous system or anterior pituitary has been demonstrated.

Mechanism of action

Parathormone mediates its effect via cell surface parathyroid hormone receptors (PTHR), located mainly on the renal epithelial cells and osteoblasts and also in some other target cells. Two subtypes of PTH receptors viz. PTH1R and PTH2R have been identified. The parathyroid hormone 1 receptor (PTH1R) is the classical PTH receptor expressed in high concentrations in bone and kidney and regulates calcium ion homeostasis. The parathyroid hormone 2 receptor (PTH2R) is expressed primarily in the central nervous system, pancreas, testis and placenta. Both receptor types belong to the superfamily of G-protein coupled receptors.

The Binding of PTH to the receptor results in increased levels of the cAMP, and inositol triphosphate and diacyl glycerol by stimulation of adenylate cyclase and phosphatidyl pathways, respectively. The accumulation of cAMP in target cells increases permeability for calcium ion. Cytosolic Ca^{++} concentration may also be increased by the actions of inositol triphosphate, which mainly stimulates release of Ca^{++} from the intracellular stores (Figure 50.6). Both cAMP and Ca^{++} serves as second messengers for the PTH in target cells and results in expression of biological reactions such as osteoclastic secretion of enzymes and acids in bone to cause bone resorption and formation of 1,25-dihydroxycholecalciferol in kidneys. Some direct actions of PTH which function independently of the 2nd messenger mechanism may also be involved in few effects of PTH.

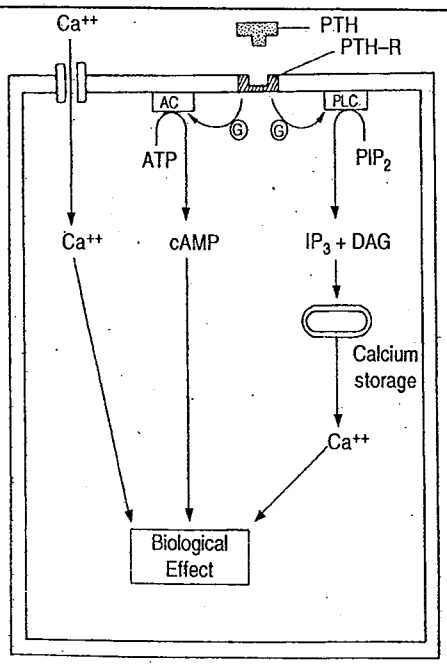


Fig. 50.6. Schematic representation of mechanism of action of parathyroid hormone.

Abbreviations: PTH = parathyroid hormone ; AC = adenylate cyclase; PLC = phospholipase C ; ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; PIP_2 = phosphatidyl inositol bisphosphate; IP_3 = inositol triphosphate ; DAG = diacyl glycerol; G = G-protein; R=receptor.

Physiological effects

The parathyroid hormone functions in calcium homeostasis by eliciting the adaptive changes which maintain a constant concentration of Ca^{++} in the extracellular fluid. It has direct effects on bone and kidney metabolism of calcium and indirect effects on GI metabolism of calcium. The net effect of parathyroid hormone is to increase plasma calcium and to lower plasma phosphate levels.

Bone: Parathyroid hormone leads to resorption of bone, causing release of calcium into the blood. It promotes osteoclastic activity and transiently depresses osteoblastic activity. These factors result in an increased absorption of bone matrix and release of calcium and phosphate from the bone.

Kidneys: Parathyroid hormone acts on renal tubules and enhances tubular reabsorption of calcium. On the other hand, PTH decreases tubular reabsorption of phosphate. These actions of PTH result in increased phosphate clearance (phosphaturia) but decreased calcium excretion. The PTH acts on kidneys and also stimulates conversion of vitamin D to its active form 1,25-dihydroxyvitamin D or calcitriol, which is then secreted into circulation and helps in absorption of Ca^{++} from the GI tract.

Gastrointestinal tract: Parathyroid hormone has no direct effect on the intestinal calcium transport. It mediates the absorption of calcium from gut indirectly through its effect on vitamin D.

DISORDERS OF PARATHYROID HORMONE

Deficiency of parathyroid hormone / Hypoparathyroidism

Deficiency of parathyroid hormone (hypoparathyroidism) has been reported in both human and veterinary medicines, but the incidence in not common in animals. Primary hypoparathyroidism mostly occurs due to iatrogenic injury or surgical removal of thyroid or parathyroid glands and may also stem from genetic or autoimmune cause. Secondary hypoparathyroidism can accompany several diseases including acute renal failure (e.g. urethral obstruction or ethylene glycol toxicity), chronic renal disease, acute pancreatitis and nutritional factors (low calcium to phosphorus ratio in diet). Cessation of PTH secretion from the parathyroid gland results in hypocalcaemia due to loss of calcium mobilisation from bones, loss of calcium retention in kidneys, and loss of calcium absorption from intestine.

The main clinical manifestations of hypoparathyroidism reflect decreased serum concentrations of calcium. Hypocalcaemia leads to neuromuscular hyperexcitability and tetany with signs of muscle cramping, lameness, irritability, stiff gait and tetanic seizures. Despite the importance of calcium in the cardiac contractility, hypocalcaemic dogs generally do not develop clinical evidence of cardiac dysfunction.

Excess of parathyroid hormone / Hyperparathyroidism

Excessive secretion of PTH by parathyroid glands occurs in both primary and secondary hyperparathyroidism. The most common cause of primary hyperparathyroidism is neoplasia of the chief cells of parathyroid gland. Hyperplasia of parathyroid gland(s) and hereditary hyperparathyroidism are less common

causes of primary hyperparathyroidism. Secondary hyperparathyroidism mostly occurs when calcium and phosphorus homeostasis becomes unbalanced (e.g. renal or nutritional factors). High PTH in blood results in increased decalcification of bone. Hypercalcaemia, hypophosphataemia and hyperphosphaturia develop due to excess of PTH and vitamin D. This leads to nephrogenic diabetes insipidus, initially increased then decreased glomerular filtration rate, nephrocalcinosis, soft tissue mineralisation and fibrous osteodystrophy of all bones.

Clinical signs of hyperparathyroidism include polydipsia, polyuria, anorexia, lethargy, depression, weakness and muscle wasting. Muscle shivering or twitching may occur in some animals. Vomiting, constipation, pancreatitis, facial swelling, renal failure and gastrointestinal ulcerations are less often reported. Some animals often remain symptomatic.

TREATMENT OF PARATHYROID HORMONE DISORDERS

Selected drugs are available for use in disorders of parathyroid hormones.

Classification

I. Drugs used in deficiency of parathyroid hormone
   e.g. parathormone and teriparatide.

II. Drugs used in excess of parathyroid hormone/Anti-parathyroid drugs
   1. Calcitonin and analogues
      e.g. calcitonin, elcatonin and cinacalcet.
   2. Vitamin D analogues
      e.g. paricalcitol and doxercalciferol.

I. Drugs Used In Deficiency of Parathyroid Hormone

Parathormone /Parathyroid hormone

Parathormone (Parathyroid hormone) is an endogenous hormone synthesised and secreted by the chief cells of the parathyroid glands. Commercially, it is prepared from the bovine glands. Preparations are adjusted to contain approximately 100 units/ml.

Pharmacological effects

The mechanism of action and biological effects of exogenous parathormone is similar to those of endogenous hormone. It elevates blood calcium level via bone (osteoclasis), kidney (enhanced calcium reabsorption) and intestine (increased calcium absorption).

Pharmacokinetics

Like other protein hormones, parathormone must be given parenterally. The SC or IM injection brings about an elevated serum calcium level within 3-4 hours, which peaks at 8-12 hours and returns to control level at 20-24 hours. The peak effect of calcium following IV parathormone is seen considerably sooner. The hormone is transported in the blood, at least in part, in the alpha-globulin fraction of plasma protein. It is metabolised mainly in the liver and kidneys. Half-life of exogenous parathyroid hormone in humans is about 20 minutes.

Side effects/Adverse effects

Overdosage with parathormone causes hypercalcaemia manifested as weakness, vomiting, diarrhoea and lack of muscle tone. Hypersensitivity reactions may occur. The development of antibodies occasionally causes intolerance and resistance making parathyroid hormone therapy ineffective.

Clinical uses

Parathyroid hormone has very limited clinical use because hypothyroidism is best treated by vitamin D and calcium supplements. Parathyroid hormone produces very slow onset of action, which precludes its use in the treatment of acute hypocalcaemia. Rarely, parathyroid hormone is used for the early control of tetany due to hypothyroidism. It has also been used for diagnosis of pseudohypoparathyroidism. If plasma calcium levels fail to rise following IV injections of parathormone, then it indicates pseudohypoparathyroidism.

Teriparatide

Teriparatide is a recombinant preparation of parathyroid hormone containing the 1-34 amino acid residues of human parathyroid hormone. It has been approved for the treatment of some forms of osteoporosis. It is used also to differentiate pseudohypoparathyroidism from true hypoparathyroidism. The most commonly reported side effects in human patients are nausea, leg cramps and dizziness. Teriparatide is not recommended in patients those are at increased risks for osteosarcoma.

II. Drugs used in excess of parathyroid hormone/ Anti-parathyroid drugs

1. Calcitonin and Analogues

Calcitonin

Calcitonin is a polypeptide hormone secreted by the parafollicular cells (C-cells) of the thyroid. Commercially it is available as calcitonin porcine, calcitonin salmon and recombinant human calcitonin. It is used occasionally to suppress the excessive bone resorption of Paget's disease and in osteoporosis. The pharmacology of calcitonin has been discussed earlier in the present Chapter.

Elcatonin

Elcatonin is a calcitonin derivative used as an anti-parathyroid agent. It is derived from eel's calcitonin by changing the S-S bond into the stable C-N bond. Its mechanism of action and pharmacological effects are similar to those of calcitonin. It prevents the absorption of calcium and decreases resorption and autolysis of bones. In addition, it inhibits renal reabsorption of calcium and phosphorus and promotes their excretion in urine. These actions help in decreasing the elevated blood calcium levels to normal. Elcatonin is mainly used for remitting or eliminating the pain caused by osteoporosis.

Cinacalcet

Cinacalcet is a calcimimetic and an anti-parathyroid drug. It mimics the action of calcium on tissues by allosteric activation of the calcium-sensing receptors which causes less production of parathyroid hormone. Cinacalcet is being used either alone or in combination with other drugs in human medicine for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease on dialysis. Cinacalcet is also indicated for the treatment of hypercalcaemia in patients with parathyroid carcinoma. Vomiting, diarrhoea, weakness and GI upsets are important side/adverse effects observed in human patients.

2. Vitamin D Analogues

Paricalcitol

Paricalcitol (19-nor-1,25-$(OH)_2$-vitamin D_2 or 19-nor-1,25-dihydroxyvitamin D_2), is an analogue of 1,25-dihydroxyergocalci-

ferol, the active form of vitamin D_2. It acts as an agonist for the vitamin D receptor and lowers the blood parathormone level. It has been recommended for the prevention and treatment of secondary hyperparathyroidism associated with chronic renal failure. The pharmacology of paricalcitol has been given in Chapter 54.

Doxercalciferol

Doxercalciferol is a synthetic form of pro-vitamin D_2. It produces pharmacological effects by decreasing the elevated levels of parathyroid hormone (PTH) and by maintaining normal calcium levels in the body. It is used mainly in the treatment of secondary hyperparathyroidism (SHPT) due to chronic kidney disease.

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CHAPTER 51 ADRENAL HORMONES

Adrenal cortex
Corticosteroids
- Biosynthesis, transport, metabolism and elimination
- Regulation of secretion
  - Glucocorticoids
  - Mineralocorticoids
- Mechanism of action
  - Glucocorticoids
  - Mineralocorticoids
- Physiological effects
  - Glucocorticoids
  - Mineralocorticoids
- Disorders of adrenocortical hormones
  - Deficiency of adrenocortical hormones/Hypoadrenocorticism
  - Excess of adrenocortical hormone/Hyperadrenocorticism
- Treatment of adrenal hormone disorders
  - Classification

Drugs used in deficiency of adrenocortical hormones
Corticosteroids
- Chemistry and structure-activity relationship
- Glucocorticoids
  - Short-acting glucocorticoids
  - Intermediate acting glucocorticoids
  - Long-acting glucocorticoids
  - Glucocorticoids used by topical and/or inhalation route
- Mineralocorticoids
- General principles in corticosteroid therapy
- Drug preparations and routes of administration
- Drugs used in excess of adrenocortical hormones (hyperadrenocorticism)
  - Inhibitors of corticosteroid biosynthesis
  - Inhibitors of glucocorticoid receptors
  - Inhibitors of aldosterone receptors
  - Inhibitors of ACTH biosynthesis and release

Adrenal (Suprarenal) glands are a pair of endocrine glands located just anterior to the kidneys. Hormones secreted by adrenal glands regulate a number of metabolic processes, which are essential for life and enable animals to function in a constantly changing environment. Each adrenal gland is divided into two separate entities, a cortex and a medulla, each of which produces different types of hormones. The outer part or adrenal cortex is derived from mesodermal tissue and contains cells which synthesise steroid hormones such as hydrocortisone, corticosterone, sex steroids and aldosterone. The inner part or adrenal medulla is derived from ectodermal tissue from the neural crest and contains the chromaffin cells those synthesise catecholamines like epinephrine and norepinephrine. The pharmacology of catecholamines has been discussed earlier in Chapter 11.

Adrenal Cortex

The adrenal cortex constitutes about 90% of the adrenal mass and can be characterised and divided into three relatively distinct zones – Zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa, the outer zone, is relatively narrow and is organised in a whorl-type cell arrangement. The zona fasciculata, the middle zone, is relatively wide and is organised with cells in columns. The zona reticularis, the inner zone, is adjacent to adrenal medulla and is intermediate in size with cells having a more random organisation (Figure 51.1). The adrenal

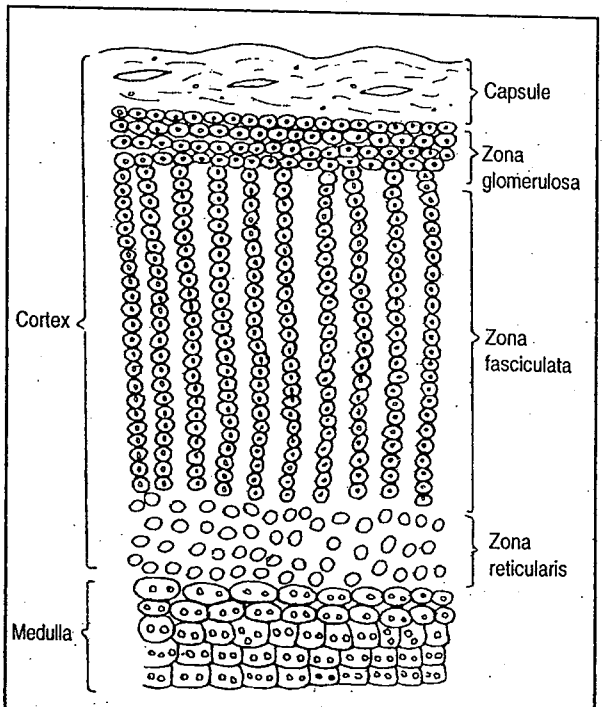


Fig. 51.1. Section of adrenal gland showing anatomically distinct zones.

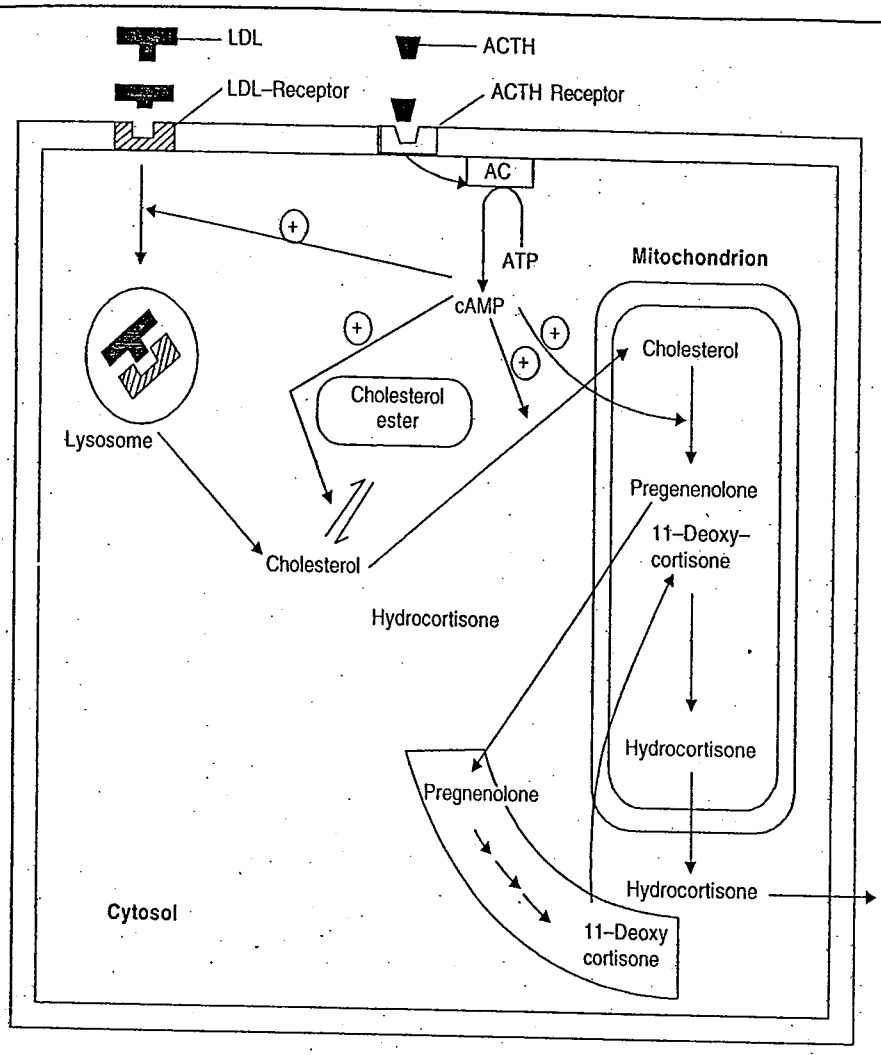


Fig. 51.2. Schematic representation of mechanism of action of ACTH and biosynthesis of hydrocortisone (See text for detail).
Abbreviations : LDL = low density lipoprotein ; ACTH = adrenocorticotropic hormone ; AC = adenylate cyclase ; ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; + = stimulation.

cortex synthesises two major types of steroid hormones viz. glucocorticoids (carbohydrate metabolism regulating) and mineralocorticoids (electrolyte balance regulating) together which are called adrenocortical hormones or corticosteroids. In addition to these, small amounts of sex steroids, especially androgenic hormones, are secreted which exhibit about the same effects in body as the male sex hormone testosterone. Glucocorticoids and sex steroids are synthesised in both the zona fasciculata and zona reticularis, whereas mineralocorticoids are mainly synthesised in the zona glomerulosa.

CORTICOSTEROIDS

The adrenal cortex produces a large number of different corticosteroid hormones. Although more than 50 different steroids have been identified, but only some such as hydrocortisone (cortisol) and corticosterone have predominantly glucocorticoid activity and aldosterone and deoxycorticosterone have predominantly mineralocorticoid activity. Among glucocorticoids, hydrocortisone predominates in man, horses, pigs, sheep and cats, and corticosterone predominates in rabbits, mice and rats. The ruminants, except sheep, are intermediate since they secrete sizable amounts of both hormones. Dogs secrete these two hormones in approximately equal amounts. The primary mineralocorticoid in most species is aldosterone. Technically, the term corticosteroid refers to both glucocorticoids and mineralocorticoids (as both are produced by the adrenal cortex), but for practical purposes it is often used as a synonym for glucocorticoids.

Biosynthesis, Transport, Metabolism and Elimination

Biosynthesis: The corticosteroids or corticoids (both glucocorticoids and mineralocorticoids) are 21 carbon compounds having a cyclopentanoperhydrophenanthrene (steroid) nucleus. These hormones are synthesised in the adrenal cortical cells from the cholesterol either obtained directly from the circulation (mainly) or synthesised from acetyl coenzyme within the cortical cells (lesser importance). The cholesterol is transported in plasma bound to low density lipoprotein (LDL) due to its water insoluble nature. The specific low-density lipoproteins receptors present on the cortical cell membrane recognize the carrier and absorb the cholesterol by process of endocytosis. After entering the cells of adrenal cortex, cholesterol either enters a biosynthetic pathway to generate corticosteroid hormones or is stored in the form of a cholesterol ester in the cytosol.

The adrenocortical cell possesses various enzyme systems within the smooth endoplasmic reticulum and mitochondria, which participate in complex biosynthetic pathways of various

corticosteroids. The first step in the synthesis of all steroid hormones from cholesterol involves cleavage of the side chain of cholesterol to form pregnenolone, a step occurring within the mitochondrion. Subsequent modifications of the steroid molecules may occur within the mitochondria or may involve movement to other compartments of the cell (Figure 51.2). The biosynthetic pathways of adrenocortical hormones and other steroid hormones are illustrated in Figure 48.2.

Transport: After biosynthesis, corticosteroids enter blood stream and circulate either in bound forms (mainly) or as free molecules. Hydrocortisone combines mainly (about 75%) with a specific binding globulin called corticosteroid binding globulin or transcortin and to lesser extent (15%) with albumin. About 10% of the hydrocortisone is transported as free molecules. The transport of aldosterone in mainly associated with albumin (50%), with only 10% associated with transcortin leaving a large amount (40%) in the free form. In both combined and free forms, hormones are transported throughout the extra cellular fluid compartment. Similar to other compounds, only free corticosteroids are biologically active and are absorbed into cells, where they initiate their specific effects. The clearance half-life of hydrocortisone is about 1 hour and that of aldosterone about 20 minutes. This is attributed to the observed difference in protein binding of these hormones within the plasma. Several physiological and patho-physiological states can influence the amount of binding proteins present in plasma and hence may affect concentration of the bound forms. For example, oestrogen produced during pregnancy results in an increase in hepatic synthesis of transcortin, whereas liver dysfunction can result in lower concentration of transcortin.

Metabolism and elimination: The adrenal steroids are metabolised mainly in liver and to a small degree in kidneys. As the first step, enzymes within hepatic cells reduce the 3-keto group to a 3-hydroxyl group, which is then conjugated especially to form glucuronides and to a lesser extent sulphates. This reduces their biological activity and renders them water soluble for passage either in urine (75%) or in faeces (25%).

Regulation of Secretion

Glucocorticoids

The output of glucocorticoids by adrenal cortex is regulated mainly by the adrenocorticotropic hormone (ACTH) synthesised and released from the anterior pituitary. The ACTH stimulates the synthesis of glucocorticoids and mineralocorticoids primarily by activating cholesterol esterase to trigger formation of free cholesterol from cholesterol ester that is bound to low density lipoprotein. This effect of ACTH on steroid synthesis is mediated by cyclic nucleotides like cAMP (mainly). The ACTH may also stimulate uptake of low-density lipoprotein, transport of cholesterol into mitochondria and binding of cholesterol to enzyme to form pregnenolone (Figure 51.2). The glucocorticoids, in turn regulate the secretion of ACTH from anterior pituitary through a negative feedback mechanism (see for detail ACTH, Chapter 49). Daily production of hydrocortisone can rise markedly (at least 10 fold) in the setting of severe stress.

Mineralocorticoids

The regulation of mineralocorticoids secretion, in contrast to glucocorticoids secretion, is not controlled by tropic hormones

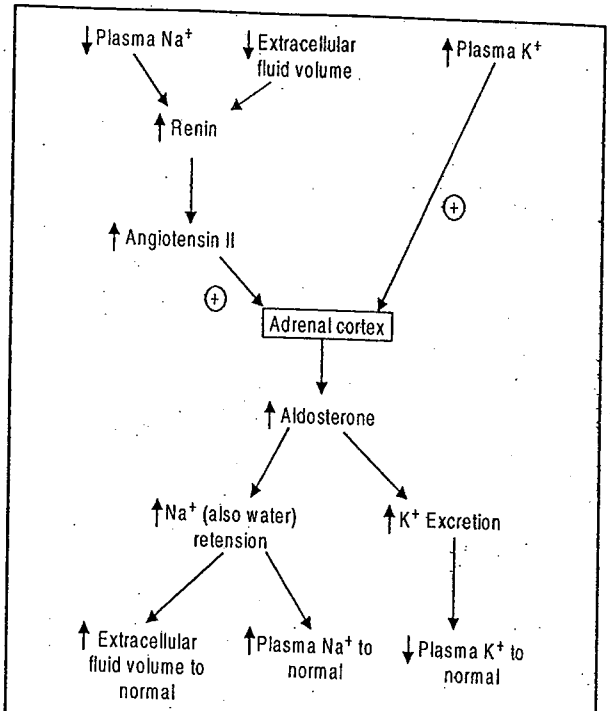


Fig. 51.3. Schematic representation of regulation and action of aldosterone.

from the pituitary. Adrenocorticotropic hormone from the anterior pituitary is necessarily required for the aldosterone secretion, but has little effect in controlling the rate of synthesis. The mineralocorticoids secretion is regulated by a complex process involving extracellular fluid electrolyte concentration, extracellular fluid volume, blood volume, arterial pressure and renal function. Increased potassium ion concentration in the ECF and increased activity of the renin-angiotensin system are by far the most potent factors causing enhanced secretion of aldosterone. A slight increase in potassium concentration and activation of renin-angiotensin system, usually in response to diminished blood flow to the kidneys, can cause several fold increase in aldosterone secretion. Enhanced synthesis of angiotensin II stimulates the zona glomerulosa to produce mineralocorticoids (angiotensin, if present on a long-term basis, also increase size of zona glomerulosa). On the other hand, increased sodium ion concentration in ECF results in decreased renin release and decrease in aldosterone secretion, and vice versa. A decline in potassium concentration also inhibits mineralocorticoids. Atrial natriuretic peptide, a 28 amino acid peptide produced by cells of the cardiac atria as well as some other sites may inhibit the production of mineralocorticoids and renin.

Mechanism of action

Glucocorticoids

Glucocorticoids are lipid-soluble steroid hormones, which diffuse through cell membranes to the cytoplasm and bind to a specific glucocorticoid receptor (GR) in target cells. The glucocorticoid receptor resides predominantly in the cytoplasm in an inactive form until it forms a complex with the hormone. After interaction with the steroid, the receptor becomes activated and undergoes a conformational change that exposes a DNA binding domain, which determines types of genes to be influenced by receptor. The steroid-receptor complex then translocates to the

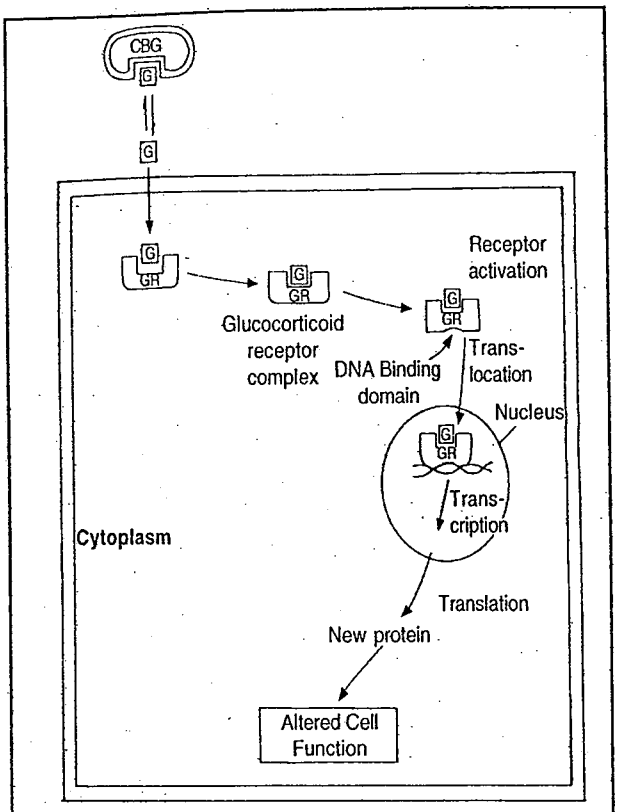


Fig. 51.4. Schematic representation of mechanism of action of glucocorticoids (See text for detail).
Abbreviations : G = glucocorticoid ; GR = glucocorticoid receptor ; CBG = corticosteroid binding globulin ; m-RNA = messenger ribonucleic acid.

nucleus and binds to glucocorticoid response elements (GRE) in the promoter region of the target genes in the DNA resulting in either induction (initiates transcription) or suppression (prevents transcription) of particular genes. The induction process, often called transactivation, involves formation of specific messenger RNAs which migrate to cytosolic ribosomes to stimulate synthesis of specific proteins (Figure 51.4). The new proteins mediate the hormonal action. Repression of specific genes, on the other hand, leads to transrepression i.e. inhibition of mRNAs and hence decreased production of certain proteins. Glucocorticoids are able to prevent the transcription of several pro-inflammatory genes, including several interleukins (IL-1B, IL-4, IL-5 and IL-8), chemokines, cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor-alpha (TNFα) genes.

The glucocorticoid receptors are very widely distributed in body and are found in virtually all cells, the number varying in different tissues. They belong to the steroid receptors superfamily that regulates DNA transcription. The glucocorticoid receptor has been cloned and has found to be made up of about 800 amino acids. There are believed to be 10 to 100 steroid responsive genes in each cell. Most of the actions of glucocorticoids are apparent after several hours as the changes in gene expression and protein synthesis require some time to become functional. However, some actions of glucocorticoids are exerted more rapidly (e.g. inhibition of ACTH release from anterior pituitary). These may be mediated by a different mechanism not involving protein synthesis. Some

Table 51.1: List of some important proteins whose synthesis is affected by glucocorticoids.

Inhibition	Induction
1. Interleukins (IL-1B, IL-4, IL-5 and IL-8)	1. Lipocortin-1
2. Chemokines.	2. Leukoprotease inhibitor 1 (SLP1)
3. Granulocyte-macrophage colony-stimulating factor (GM-CSF)	3. Beta$_2$-adrenceptor
4. Tumour necrosis factor-alpha (TNFα)	4. Angiotensin converting enzyme (ACE)
5. Natural killer$_1$ receptor	5. Na^+-K^+-ATPase (by mineralocorticoid).
6. Cytokinase-2	6. Glucose-6-phosphatase
7. Phosphlipase-2	
8. Collagenase	
9. Stromelysin	
10. Inducible nitric oxide synthase	
11. Endothelin-1	

important proteins whose synthesis is targeted by glucocorticoids are enlisted in Table 51.1.

Mineralocorticoids

Mineralocorticoids, like glucocorticoids and other steroids, bind to specific intracellular receptors and activate the transcription of discrete sets of genes within target tissues. However, unlike the glucocorticoid receptors which occur in most tissues, mineralocorticoid receptors are found only in a few target cells such as kidneys, salivary glands, sweat gland and in transporting epithelia of colon and bladder. Further, in comparison with glucocorticoids, mineralocorticoids induce synthesis of a limited number of proteins, the most important of which is Na^+-K^+-ATPase.

In general, mineralocorticoids initiate transcription of specific proteins which produce an early effect and a late effect. In early effect, mineralocorticoids mainly cause increase in the number of renal tubular cells through an unidentified protein mediator. In late phase, mineralocorticoids increase number of Na^+-K^+-ATPase, a protein found in the basolateral membrane of the tubular cells which generates electrochemical gradient and drives monovalent cations (i.e. Na^+ and K^+) through their respective membrane channels (see for detail, Chapter 38). There is also evidence for a rapid non-genomic effect of aldosterone on Na^+ influx through an action on the Na^+/H^+ exchanger in the apical membrane.

Physiological Effects

Glucocorticoids

Glucocorticoids affect a wide range of activities in the body. They prepare body to withstand effect of all kinds of noxious stimuli and stress. They have some direct and some permissive effects (i.e. facilitates other hormones to exert effects). The permissive effects most likely reflect steroid-induced changes in protein synthesis that, in turn, modify tissue responsiveness. The biological effects of glucocorticoids differ with dose i.e. some changes occur with physiological concentrations, whereas others

transpire with supraphysiological or pharmacological levels. However, distinctions between physiological and pharmacological effects can be difficult, so both types are considered together.

The biological effects of glucocorticoids can be studied under following headings:

Effects on metabolism

Carbohydrate metabolism: The name "glucocorticoid" derives from early observations that these hormones were involved in glucose metabolism. Glucocorticoids have minimal effect on blood glucose concentrations in the normal fed animal. In the fasted state, they stimulate several processes which collectively serve to increase and maintain normal concentrations of glucose in blood. These processes may include stimulation of gluconeogenesis, inhibition of peripheral glucose utilisation and increasing tissue stores of glycogen. Glucocorticoids stimulate gluconeogenesis by increasing synthesis of all enzymes required to convert amino acids into glucose in liver cells and by mobilising amino acids from extrahepatic tissue, mainly from muscle. Decreased peripheral tissue utilisation of glucose occurs mainly due to glucocorticoid induced reduced affinity of cellular receptors for insulin (anti-insulin effect). Glucocorticoids promote glycogen deposition in liver mainly by inducing hepatic glycogen synthetase and also through gluconeogenesis. Both the increased rate of gluconeogenesis and the moderate reduction in the rate of glucose utilisation by cells cause blood glucose concentrations to increase that could lead to glucosuria, a condition called adrenal diabetes. Overall, the function of glucocorticoids appears to be oriented to maintaining blood glucose levels during starvation and stressful conditions, so that brain continues to get nutrition even in adverse conditions.

Protein metabolism: Glucocorticoids reduce protein stores in essentially all body cells except those of liver by both decreased protein synthesis and increased protein breakdown. The increased protein catabolism leads to negative nitrogen balance, elevated amino acids in blood and an increase in urinary elimination of nitrogen and uric acid. Enhanced breakdown of muscle proteins may lead to muscle wasting, weakness, thinning of skin, delayed wound healing and loss of osteoid from bone. The transport of amino acids across all extrahepatic cell membranes decreases, but hepatic transport of amino acids increases for conversion of amino acids to glucose (gluconeogenesis) and also for synthesis of liver and plasma proteins. Inhibition of synthesis of proteins by hydrocortisone and other glucocorticoids may lead to reduce growth in young animals.

Lipid metabolism: Glucocorticoids promote lipolysis and generate free fatty acids those along with amino acids serve as substrates for hepatic glycogen synthesis. Hydrocortisone also enhances oxidation of fatty acids in cells and inhibits long chain fatty acids. The increased mobilisation of fat combined with increased oxidation of fatty acids in cells shifts the metabolic systems of cells during starvation or other stresses from utilisation of glucose for energy to utilisation of fatty acids. The action of glucocorticoids on lipid metabolism is mainly permissive in nature through facilitation of adipokinetic hormones such as catecholamines, glucagon, growth hormone and thyroxine. In absence of lipolytic hormones, glucocorticoids have virtually insignificant effect on the rate of lipolysis by adipocytes.

Calcium metabolism: Glucocorticoids inhibit intestinal absorption and enhance renal excretion of Ca^{++}. There is also loss of calcium from bone indirectly due to loss of osteoid (decreased formation and increased resorption). Glucocorticoids also increase parathormone secretion, which in turn increases osteoclast-mediated bone resorption. Spongy bones (e.g. vertebrae and ribs) are more sensitive to the effects of glucocorticoids. The net effect of glucocorticoids on calcium homeostasis is decrease in total body calcium stores.

Anti-inflammatory and immunosuppressive effects

Glucocorticoids possess anti-inflammatory and immunosuppressive effects. Because the anti-inflammatory and immunosuppressive effects of glucocorticoids reflect specific actions on white blood cells, these effects are usually interlinked. The anti-inflammatory and immunosuppressive effect of glucocorticoids are evident generally when steroids are present in high concentrations (greater than those found in normal physiological state) or given in pharmacological doses.

Anti-inflammatory effects: Glucocorticoids suppress all types of inflammatory reactions whether caused by invading pathogens, by chemical, thermal or mechanical stimuli, or by hypersensitivity or autoimmune diseases. Their actions are non-specific and cover all components and stages of inflammation. In early stages of inflammation, they inhibit responses like redness, pain, heat, swelling, leucocytes migration and phagocytic activity of leucocytes. In late or chronic inflammation, glucocorticoids cause reduced fibroblast activity, collagen synthesis, capillary permeability, tissue repair and ultimately scar formation. Although glucocorticoids cause early resolution of the chronic inflammation, they generally delay healing and repair; peptic ulcers may perforate asymptomatically.

Many of the anti-inflammatory processes involve action of glucocorticoids on inflammatory cells and inflammatory mediators. Specifically, glucocorticoids inhibit the enzyme phospholipase A_2 via induction of an anti-inflammatory protein lipocortin-1 (annexin-1). This blocks release of arachidonic acid, the precursor of prostaglandins and leukotrienes, and also synthesis of platelet activating factor. Glucocorticoids are also reported to directly suppress cyclooxygenase (COX) expression; the effect being much like that of NSAIDs, potentiating the anti-inflammatory effect produced at the level of phospholipase A_2 enzyme. Glucocorticoids also cause stabilisation of plasma and lysosomal membranes resulting in decreased release of autacoids and proteolytic enzymes. Although glucocorticoids interfere at several steps in the inflammatory response (see Table 51.2), the most important overall mechanism appears to be the limitation of recruitment/movement of inflammatory cells at local site. These changes in cell distribution decrease inflammation due to less availability of inflammatory cells. In addition, the phagocytic activity of inflammatory cells may be diminished. Macrophages are considered more sensitive to glucocorticoids than the neutrophils. Glucocorticoids down-regulates the expression of Fc receptors on macrophages, causing decreased phagocytosis of opsonised cells. Glucocorticoids also lower fever mainly because they reduce the release of interleukin-1 from leucocytes, which is one of the principal excitant to the hypothalamic temperature control system.

Immunosuppressive effects: Glucocorticoids exert profound effect on specific host-immune responses. They suppress all types of hypersensitisation and allergic phenomenon and can impair immunological competence. The immunosuppressive actions of

Table 51.2: Mechanisms of anti-inflammatory and immunosuppressive effects of glucocorticoids.

Mechanism	Action
1. Stabilisation of plasma and lysosomal membranes.	Decreased release of autacoids (e.g. histamine) and proteolytic enzymes resulting in diminished vascular permeability and tissue destruction.
2. Induction of lipocortin in macrophages, endothelium and fibroblasts.	Inhibition of phospholipase A_2 causing decreased production of prostaglandins, leukotrienes and platelet activating factor.
3. Inhibition of genes for cytokines in macrophages, endothelial cells and lymphocytes.	Decreased production of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-8, TNFα, GM-CSF and γ-interferon resulting in suppression of fibroblast proliferation and T-lymphocyte function. Chemotaxis is also interfered.
4. Decreased production of ELAM-1 and ICAM-I in endothelial cells.	Adhesion and localisation of leucocytes is interfered.
5. Decreased production of acute phase reactants from macrophages and endothelial cells.	Complement fixation is interfered.
6. Inhibition of IgE mediated histamine and LTC_4 release from basophils.	Inhibition of antigen-antibody mediated effects.
7. Decreased production of collagenase and stromelysin.	Prevention of tissue destruction.
8. Inhibition of inducible form of nitric oxide synthase.	Decreased production of nitric oxide (NO) resulting in decreased vasodilatation.

Abbreviations: IL = interleukin; TNFα = tumour necrosis factor alpha; GM-CSF = granulocyte macrophage colony stimulating factor; ELAM-1 = endothelial leucocyte adhesion molecule-1; ICAM-1 = intracellular adhesion molecule-1; IgE = immunoglobulin E; LTC_4 = leukotriene C_4.

glucocorticoids are more pronounced on cell-mediated immunity in which T cells are principally involved than the humoral immunity involving B cells. They act mainly by inhibiting genes which code for the cytokines, the most important of which is IL-2. Smaller cytokine production reduces the T cell proliferation. Glucocorticoids also suppress the humoral immunity, causing B cells to express smaller amounts of IL-2 and of IL-2 receptors. This diminishes both B cell clone expansion and antibody synthesis. The impairment of antibody production or complement function is less seen at low concentrations or at therapeutic doses, but is pronounced at high levels. It has been shown that high concentrations of glucocorticoid interfere with practically every step in the immunological responses from the initial interaction and processing of antigens by cells of the reticuloendothelial system to the antibody production. It is proposed that inhibition of immune system by glucocorticoids protect the organism from potentially life-threatening consequences of a full blown inflammatory reaction. However, high levels of glucocorticoids reduce resistance to bacteria, viruses and fungi, which favours spread of infection. This occurs mainly because the capacity of defensive cells to kill microorganisms is suppressed by glucocorticoids. High concentrations of glucocorticoids also cause significant atrophy of all lymphoid tissues throughout the body, which in turn decreases the output of both T cells and antibodies from lymphoid tissue.

Out of various factors involved in immunosuppression, glucocorticoids exert important effect on cytokine production. Factors which are inhibited include interleukins (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-8), tumour necrosis factor alpha (TNF-α), interferon gamma, granulocyte/monocyte colony stimulating factor (GM-CSF). Multiple cytokines also appear to regulate the hypothalamic-pituitary-adrenal (HPA) axis, which has an intimate relationship with the immune system. Among these, IL-1 interacts directly with the pituitary and hypothalamus to increase release of ACTH and CRH, respectively, and also with the adrenal gland to increase release of glucocorticoids. Various cytokines and other factors involved in biological responses have been described in Chapter 56.

Effects on body systems

Cardiovascular system: Glucocorticoids decrease capillary permeability and maintain tone of arterioles. They enhance vascular reactivity to other vasoactive substances (permissive effect). Hypoadrenalism is generally associated with hypotension, decreased cardiac output and reduced response to vasoconstrictors such as epinephrine and angiotensin II. Conversely, hypertension is seen in patients with excessive glucocorticoid secretion. The elevated pressor response to epinephrine could be explained by studies, which have shown that glucocorticoids increase expression of adrenergic receptors in the vascular wall.

Haematopoietic system: Glucocorticoids decrease the number of eosinophils, monocytes and lymphocytes in blood but may cause neutrophilia. Prolonged therapy with glucocorticoids has been associated with involution of lymph nodes, thymus and spleen. The thymus derived lymphocytes (T-cells) decrease proportionately more than the lymphocytes derived from bone marrow (B-cells). The neutrophilia induced by glucocorticoids is the result of an increase in number of neutrophils entering the bloodstream from bone marrow and from diminished rate of removal from the circulation. Glucocorticoids tend to increase the number of red blood cells (polycythemia) mainly due to enhanced production of erythrocytes and a diminished erythrophagocytosis. Therefore, anaemia often results in absence of glucocorticoids.

Central nervous system: Glucocorticoids exert a number of indirect effects on the CNS through maintenance of blood pressure, glucose concentration and electrolytes concentration. They decrease

formation of CSF, which results in reduction of intracranial pressure. Glucocorticoids have direct effects on the CNS affecting mood, behaviour and brain excitability (mild euphoria). Behavioural changes such as well-being and appetite improvement are frequently manifested following glucocorticoid therapy seen in terminal conditions as cancer. On the other hand, deficiency of glucocorticoids may lead to apathy, depression and occasionally psychosis as seen in human patients of Addison's disease.

Musculoskeletal system: Glucocorticoids are catabolic to connective tissue, muscle and bone. Optimum concentrations of glucocorticoids are required for the normal functions of skeletal muscle as muscular weakness occurs in both hypo- and hyper-adrenocorticism. Hypoadrenocorticism may cause muscle weakness and diminish work capacity primarily due to hypodynamic circulation (hypotension). Hyperadrenocorticism also causes muscular weakness, but it occurs due to increased protein breakdown and decreased glucose utilisation in muscles. Glucocorticoids can lead to a loss of bone density through a variety of mechanisms i.e. suppression of osteoblast formation (decreases new bone formation) and stimulation of osteoclast-mediated bone resorption. Chronic glucocorticoid therapy, therefore, may lead to osteoporosis and cause easy bone fractures. There is enhanced excretion of calcium and phosphorus with a concomitant decrease in gastrointestinal absorption of these two agents.

Endocrine functions: Excess of glucocorticoids may suppress production and release of ACTH and/or CRH through a negative feedback inhibition. Excess of glucocorticoids may also inhibit production of TSH; however, growth hormone production is increased. Glucocorticoids exert an androgenic action which appears in both sexes following prolonged therapy. Decrease gonadotropins secretions have been reported in bitches, which may account for prolonged anoestrus seen in Cushing's syndrome. Glucocorticoids also increase parathormone secretion, which in turn increases osteoclast mediated bone resorption.

Other systems: High concentrations of glucocorticoids may stimulate gastric acid and pepsin production and may aggravate peptic ulcers. Glucocorticoids are reported to have permissive effects on beta$_2$-adrenoceptors, promoting bronchodilation. In some species (e.g. ruminants and horses), glucocorticoids may cause luteolysis and can induce uterine contractions.

Electrolyte and water balance: Glucocorticoids exert effects on fluid and electrolyte balance, largely due to permissive effects on tubular function and actions which maintain glomerular filtration (glucocorticoids, but not mineralocorticoids, maintain normal glomerular filtration rate). Glucocorticoids also enhance secretory activity of renal tubules. Hydrocortisone deficiency can lead to profound dehydration, volume depletion and shock, whereas hydrocortisone excess has been associated with water accumulation in humans and dogs.

Mineralocorticoids

The principal mineralocorticoid action is enhancement of Na^+ reabsorption in the distal convoluted tubule in kidneys (see also Chapter 38). There is an associated increase in water uptake, and K^+ and H^+ excretion. Similar actions on cation transport are exerted in other tissues also (e.g. colon, salivary glands, stomach and sweat glands). As discussed earlier, the action of mineralocorticoids is expressed mainly by gene mediated increased transcription of m-RNA, which directs synthesis of proteins (e.g. Na^+-K^+-ATPase and other aldosterone induced proteins). Because of the time taken to induce protein synthesis, mineralocorticoids have a latency of action of about 1-2 hours.

DISORDERS OF ADRENOCORTICAL HORMONES

Deficiency of Adrenocortical Hormones/Hypoadrenocorticism

A deficiency of adrenocortical hormones (Hypoadrenocorticism or Addison's disease) results from failure of the adrenal cortices to produce adrenocortical hormones. In veterinary medicine, it is seen mostly in young to middle aged dogs and occasionally in horses. The disease occurs not only due to shortage of glucocorticoids, but also a deficiency of mineralocorticoids. Since both corticosteroids are life sustaining, management of this disease is important.

Hypoadrenocorticism may be classified as primary and secondary hypoadrenocorticism. The primary hypoadrenocorticism occurs due to defective functioning of the adrenal cortices, while secondary hypoadrenocorticism occurs due to deficiency of ACTH. Primary hypoadrenocorticism mainly occurs due to atrophy of the adrenal cortices (bilateral idiopathic adrenocortical atrophy) caused by autoimmune reactions. Destruction of both adrenal cortices by infectious diseases, infarctions or tumours, and extensive bilateral adrenal cortical haemorrhage may occasionally lead to primary hypoadrenocorticism. Pharmacological inhibition of adrenal cortex by excessive administration of synthetic glucocorticoids or overdose of mitotane may also cause primary hypoadrenocorticism. The plasma hydrocortisone levels are below the resting reference range and there is little or no increase in blood hydrocortisone level in primary hypoadrenocorticism after ACTH administration.

Many of the functional disturbances of chronic adrenal insufficiencies are not highly specific and may include clinical manifestations of abdominal pain, anorexia, vomiting, diarrhoea and weight loss. Decreased production of glucocorticoids may lead to development of moderate hypoglycaemia due to suppressed gluconeogenesis and enhanced sensitivity to insulin. In the acute condition of Addisonian crisis, cardiovascular complications may lead to circulatory collapse, severe depression, unconsciousness, coma and death. A reduction in secretion of aldosterone, the principal mineralocorticoid, results in marked alterations in serum levels of potassium, sodium and chloride. Potassium excretion by the kidneys is reduced, whereas sodium and chloride loss from renal tubules is enhanced. Mineralocorticoid activity is generally maintained in pituitary dependent secondary hypoadrenocorticism, as ACTH is necessary for stimulation of synthesis but not secretion of mineralocorticoids.

Excess of Adrenocortical Hormone / Hyperadrenocorticism

Excess of adrenocortical hormones (Hyperadrenocorticism, hypercortisolism or Cushing's disease) generally results from either a hydrocortisone-secreting tumour of one adrenal cortex or general hyperplasia of both adrenal cortices. The hyperplasia of adrenal cortices in turn is generally caused by increased secretion of ACTH (secondary hyperadrenocorticism). Pharmacological elevation of glucocorticoids levels by excessive and prolonged administration of glucocorticoids may also lead to signs similar to those of hypersecretion by adrenal cortex.

Hyperadrenocorticism results in many clinical and pathological manifestations of an elevated level of glucocorticoids. Dogs develop a spectrum of clinical signs and laboratory

abnormalities as a result of the combined glucogenic, lipolytic, protein catabolic and anti-inflammatory effects of the glucocorticoid hormones on many organ systems. The clinical signs vary, but generally include polydipsia, polyuria, polyphagia, pendulous abdomen, bilateral alopecia, hyperpigmentation of skin, atrophy and thinness of skin with visible vessels, muscle weakness and atrophy, and lethargy. Central nervous system dysfunctions, behaviour disorders (decreased responsiveness to attention or diminished enthusiasm of greeting behaviour), convulsions and sleep-wake cycle disturbances may also be observed. Clinical signs in horses may include hirsutism, laminitis, polydipsia and polyuria. Hyperadrenocorticism is rarely seen in cats.

Overproduction of mineralocorticoids, a condition called hyperaldosteronism or aldosteronism, usually does not occur in secondary hyperadrenocorticism. As secondary hyperadrenocorticism accounts for about 80% of natural cases of Cushing's disease, overproduction in mineralocorticoids has not been documented frequently in animals. Occasionally, a small tumour of the *zona granulosa* cells occurs and secretes large amounts of aldosterone, which is called primary aldosteronism. Secondary (Hyper-reninemic) hyperaldosteronism indirectly results from excess secretion of renin, as the body's aldosterone production is normally regulated by renin levels. Excess aldosterone may lead to hypokalaemia, slight increase in extracellular fluid and blood volumes, and very slight increase in plasma sodium concentration. Excess aldosterone may lead to decreased plasma renin concentration. An occasional adrenocortical tumour secretes excessive quantities of androgens which cause intense masculinising effects in females. Adrenal carcinoma is an extremely rare cause of primary hyperaldosteronism.

TREATMENT OF ADRENAL HORMONE DISORDERS

Adrenal disorders can cause adrenal glands to make too much or too less of hormones. A wide variety of drugs are available for the treatment of adrenal disorders. The type of treatment chosen depends on several factors including the kind and degree of adrenal disorder, types of drug available and condition of patient.

Classification

Drugs used in the treatment of adrenal hormones disorders are broadly divided into two classes: Drugs used in deficiency of adrenal hormones (hypoadrenocorticism) and drugs used in excess of adrenal hormones (hyperadrenocorticism).

I. Drugs used in deficiency of adrenal hormones (Hypoadrenocorticism)
   A. Corticosteroids
      1. Glucocorticoids
         a. Short acting glucocorticoids (biological half-life <12 hours)
            e.g. hydrocortisone and cortisone.
         b. Intermediate acting glucocorticoids (biological half-life 12-36 hours)
            e.g. prednisolone, prednisone, methylprednisolone, triamcilone and isoflupredone.
         c. Long-acting glucocorticoids (biological half-life >36 hours)
            e.g. dexamethasone, betamethasone, flumetasone and paramethasone.
         d. Topical and inhalant glucocorticoids
            e.g. beclometasone, budesonide, mometasone, clobetasone and flumetasone.
      2. Mineralocorticoids
         e.g. fludrocortisone, desoxycorticosterone and aldosterone.

II. Drugs used in excess of adrenal hormones (Hyperadrenocorticism)
   1. Inhibitors of corticosteroid biosynthesis
      e.g. mitotane, ketoconazole, metyrapone, aminoglutethimide and trilostane.
   2. Inhibitors of glucocorticoid receptors
      e.g. mifepristone.
   3. Inhibitors of aldosterone receptors
      e.g. spironolactone, eplerenone, canrenone and potassium canrenoate.
   4. Inhibitors of ACTH biosynthesis and release
      e.g. bromocriptine, selegiline, pergolide and cyproheptadine.

I. DRUGS USED IN DEFICIENCY OF ADRENOCORTICAL HORMONES (HYPOADRENOCORTICISM)

A. CORTICOSTEROIDS

Conventionally the term 'corticosteroid' encompasses the steroid hormones synthesised and secreted by the three zones of adrenal cortex and includes both glucocorticoids and mineralocorticoids. However in pharmacology the term corticosteroid or corticoid includes not only natural glucocorticoids and mineralocorticoids but also their synthetic analogues. Corticosteroids are one of the most commonly prescribed classes of medication in both human and veterinary medicines. Corticosteroids are used in physiological doses for replacement therapy in adrenal insufficiency. These are also used in pharmacological doses primarily for their anti-inflammatory and immunosuppressive properties and for treatment of shock.

Chemistry and Structure-Activity Relationship

The corticosteroids (glucocorticoids and mineralocorticoids) are 21 carbon compounds having cyclopentanoperhydrophenanthrene (steroid) nucleus. Chemical modification to the hydrocortisone molecule has generated more than 50 different compounds, which differ primarily in their duration of action, mineralocorticoid activity and anti-inflammatory potency. Changes in chemical structures may bring about changes in specificity and/or potency as a result of changes in affinity and intrinsic activity at corticosteroid receptors, alteration in pharmacokinetics or membrane permeability. A vast array of different steroid preparations is therefore available for oral, parenteral, topical and inhalation use. Despite the multiplicity of biological effects produced by corticosteroids, following generalisations can be made on the basis of general steroid structure given in Figure 51.5:

1. The presence of 4-5 double bond and 3-keto group are essential for both glucocorticoid and mineralocorticoid activity.
2. An 11β-hydroxyl group on ring C is required for glucocorticoid activity but not mineralocorticoid activity. The presence of a keto group abolishes corticoid activity. The 11-keto group has to be converted to 11-OH for action.
3. The presence of OH at C-17 increases glucocorticoid activity. While steroids without the 17-OH (e.g.

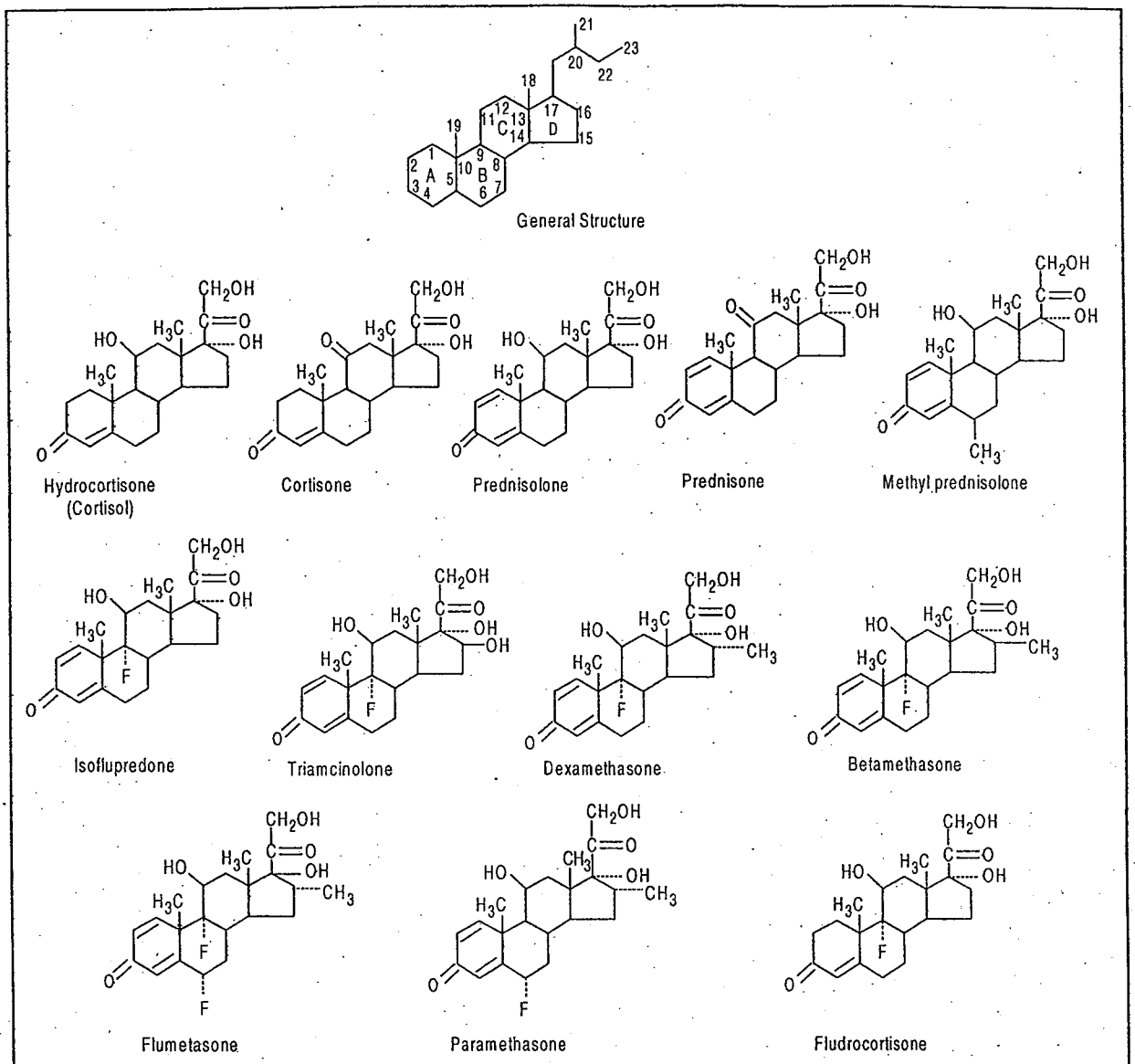


Fig. 51.5. Chemical structures of important synthetic corticosteroids. Groups attached to the steroid ring are shown either in solid lines (forward in two dimentional representation) and are designated β or in broken line (behind in two dimentional representation) and are designated α.

corticosterone) have appreciable glucocorticoid activity, the 17-OH group gives optimal potency.

4. Addition of 1-2 double bond increases the ratio of glucocorticoid to mineralocorticoid effects and increases the duration of action (e.g. prednisone and prednisolone).
5. Fluorination at the C-9 position increases both mineralocorticoid and glucocorticoid potencies. Fludrocortisone has enhanced activity at the glucocorticoid receptor but even greater activity at the mineralocorticoid receptor.
6. Methylation at the C-16 position eliminates mineralocorticoid activity.
7. Halogenation or methylation at position 6 or 9 potentiates corticosteroid activity, especially glucocorticoid activity.

Other modifications: A number of modifications convert the glucocorticoids to more lipophilic molecules with enhanced topical to systemic potency ratios. Examples include the introduction of an acetonide between hydroxyl groups at C16, C17; esterification of hydroxyl groups with valerate at C17; esterification of hydroxyl groups with propionate at C17 and C21; and substitution of the hydroxyl group at C21 with chlorine.

1. Glucocorticoids

Glucocorticoids are among the most frequently used and misused drugs in both human and veterinary medicines. They have numerous applications from physiological replacement therapy to anti-inflammatory and immunosuppressive actions. When glucocorticoids are indicated, these compounds should be used to achieve strict therapeutic goal at the lowest dose and shortest duration possible to minimise the adverse effects.

1. Short-Acting Glucocorticoids

Hydrocortisone

Hydrocortisone (Cortisol) is the principal glucocorticoid produced by the adrenal gland. Commercially available hydrocortisone is synthetic 17-hydrocortiscosterone, a replica of the compound naturally produced by the adrenal cortex.

Hydrocortisone occurs as an odourless, white to practically white crystalline powder and is used orally. Hydrocortisone is available also in various salt preparations for parenteral and oral routes and for topical application. Although it is not frequently used for systemic therapy because of the short duration of action and low potency, hydrocortisone is often described as the prototype drug.

Pharmacological effects

The mechanism of action and pharmacological effects of exogenous hydrocortisone are essentially similar to those of physiological effects produced by the endogenous glucocorticoid hormone. In addition to primary glucocorticoid, it has significant mineralocorticoid activity also.

Pharmacokinetics

Hydrocortisone is effective by the oral route. Water-soluble esters such as hydrocortisone hemisuccinate/sodium succinate can be given IM or IV route and they act rapidly and achieve high concentrations in tissues. Insoluble esters like hydrocortisone acetate cannot be injected IV, but can be administered via intra-articular, intramuscular, intrabursal, intralesional, intrasynovial or soft tissue injection to produce more prolonged effects.

After oral administration, hydrocortisone undergoes first pass metabolism in humans and has low oral : parenteral activity ratio. Hydrocortisone is 90% bound to plasma proteins, mostly to a specific corticosteroid binding globulin (transcortin) as well as to albumin. The hydrocortisone is metabolised rapidly by the hepatic microsomal enzymes mainly by reduction of 4-5 double bond, hydroxylation of 3-keto group, and reduction of 20-keto to 20-hydroxy form. The initial metabolites are further conjugated with glucuronic acid or sulphate and are excreted in urine. The plasma half-life of hydrocortisone in dogs is about 55 minutes and in humans is 1.5 hours, but the biological half-life is longer (<12 hours) because of action through intracellular receptors and regulation of protein synthesis.

Side effects/Adverse effects

The side/adverse effects of hydrocortisone and other glucocorticoids are dose and time dependent. Many side effects are extension of the physiological effects described earlier and are similar to symptoms of hyperadrenocorticism. In general, dogs are more susceptible to these compounds than cats. Important side/adverse effects of hydrocortisone and other glucocorticoids include polydipsia, polyuria, hyperglycaemia and in excess glucosuria. Protein catabolism may lead to muscle wasting, muscle atrophy and delayed wound healing. The lipolytic effect of drug is accompanied by redistribution of body fat leading to the characteristic pendulous abdomen in dogs. Prolonged administration of hydrocortisone and other corticosteroids may result in hepatomegaly with concurrent raised serum-hepatic enzyme concentrations. Reduced GI motility, thinning of gastric mucosa and reduced mucus production may develop. Altered calcium metabolism with prolonged treatment may lead to osteoporosis and bone fractures. Immunosuppressive action of glucocorticoids may lead to increased risk of infections.

Hydrocortisone or other glucocorticoids when given short-term are unlikely to cause harmful effects even in massive doses. However, chronic use of drug in high doses can lead to serious adverse effects as mentioned above. Prolonged use of drug suppresses the hypothalamic-pituitary-adrenal axis via a feedback inhibition on anterior pituitary and hypothalamus. Chronic suppression of the axis can result in iatrogenic hypoadrenocorticism on abrupt discontinuation of exogenous steroid. These and other possible adverse effects of hydrocortisone and other steroids are listed in Table 51.4.

Contraindications and precautions

Because glucocorticoids produce profound actions on virtually all systems of the body, their use should be restricted to achieve strict therapeutic goals with the lowest dose and shortest duration possible. Glucocorticoids are direct antagonists of insulin; therefore, they exacerbate diabetes mellitus and should not be used in animals with this disease. Hydrocortisone should not be used in animals in viral, bacterial and fungal infections because it can aggravate infection due to its immunosuppressive properties (a single anti-inflammatory dose is unlikely to be harmful). Other important contraindications of hydrocortisone include peptic ulcer, osteoporosis, congestive heart failure and epilepsy (glucocorticoids lower threshold to seizure). Hydrocortisone should not be used in pregnancy because it may induce parturition or abortion and may cause foetal abnormalities (e.g. cleft palate). Glucocorticoids are contraindicated in osteochondrosis dissecans because increased cartilage flap formation and stripping of cartilage from bone have been described.

Termination of hydrocortisone and other glucocorticoids therapy in chronically treated patient should be performed gradually over several weeks because acute adrenal insufficiency may result from rapid withdrawal of glucocorticoid. If a patient on steroid therapy develops an infection, the glucocorticoid should not be discontinued despite its propensity to weaken host defence. Rather, the dose may be increased to meet the stress of infection. Glucocorticoids should not be used for the treatment of laminitis in horses. Glucocorticoids should not be administered in conjunctiva with a vaccine or a toxoid as they may diminish immune response. In case of live attenuated vaccines, a virus replication may be augmented by these drugs.

Drug interactions

Hydrocortisone and other glucocorticoids show interactions with a number of drugs. Hydrocortisone may increase insulin requirements in diabetes mellitus. Administration of non-steroidal anti-inflammatory drugs (NSAIDs) along with glucocorticoids may increase the risk of gastrointestinal ulceration. When a glucocorticoid is used concurrently with digitalis, an increased risk of digitalis toxicity may occur should hypokalaemia develop. Phenobarbital sodium, phenytoin and rifampicin may increase the metabolism of glucocorticoids. Concomitant administration of hydrocortisone with oestrogens may potentiate activity of corticosteroids.

Due to wide drug interactions, combination of any drug with corticosteroids in fixed dose formulation for internal use is banned.

Clinical uses

Hydrocortisone is useful clinically for the replacement therapy in deficiency states like hypoadrenocorticism. For treatment of acute hypoadrenocorticism, if a mineralocorticoid such as fludrocortisone or desoxycorticosterone (DOCP) is unavailable, hydrocortisone may provide adequate mineralocorticoid activity. Hydrocortisone is also useful in shock, allergic emergencies, heat stroke and status asthmaticus in which alleviation of the inflammatory response is of primary concern. In such potentially fatal conditions, intensive short-time glucocorticoid use can decrease morbidity and mortality. As the absorption from SC or

Table 51.3: List of important indications for glucocorticoid use.

	System/Disease	Condition(s)
1.	Endocrine	a. Acute adrenal insufficiency b. Chronic adrenal insufficiency c. Adrenal-pituitary axis functioning
2.	Dermatological	a. Canine atopy/Flea allergy dermatitis b. Parasitic dermatitis c. Drug reactions d. Immune mediated skin diseases – pemphigus complex, systemic lupus erythematosus, discoid lupus erythematosus. e. Pyotraumatic dermatitis f. Allergic dermatitis and urticaria g. Non-specific eczema.
3.	Respiratory	a. Chronic obstructive pulmonary disease (COPD) b. Feline asthma syndrome c. Chronic/Allergic bronchitis d. Inhalation injury e. Pulmonary eosinophilic or macrophage infiltrates. f. Heart worm sensitivity g. Aspiration pneumonia h. Pulmonary oedema from drowning
4.	Gastrointestinal	a. Colitis/Ulcerative colitis b. Inflammatory bowel disease c. Non-suppurative cholangitis/ Cholangiohepatitis. d. Feline gingivitis/Stomatitis e. Lymphangiectasia
5.	Nervous	a. Cerebral oedema/Hydrocephalus b. Acute trauma c. Intervertebral disk disease d. Granulomatous meningoence-phalitis e. Non-infectious meningitis f. Spondylopathy
6.	Ophthalmological	a. Allergic conjunctivitis b. Iritis c. Vestibular disorders d. Acute uveitis e. Optic neuritis f. Keratitis
7.	Cardiovascular	a. Acute circulatory failure b. Shock/haemorrhagic shock c. Transfusion reactions
8.	Haematopoictic	a. Immune mediated haemolytic anaemia b. Immune mediated thrombocytopenia c. Immune mediated neutropenia
9.	Musculo-skeletal	a. Immune mediated arthritis (rheumatoid arthritis)/traumatic arthritis b. Bursitis, tendinitis c. Polymyositis d. Osteoarthritis e. Immune mediated myasthenia gravis f. Synovitis, capsulitis
10.	Reproduction/ lactation	a. Induction of parturition b. Termination of pregnancy/abortion c. Bovine mastitis
11.	Neoplasia	a. Leukaemia b. CNS tumours c. Lymphosarcoma d. Mast-cell tumours e. Multiple myeloma
12.	Otic	a. Otitis externa b. Idiopathic hyperplastic otitis externa
13.	Severe allergic reactions	a. Anaphylaxis b. Angioneurotic oedema c. Serum sickness
14.	Multisystemic	a. Heat stroke b. Bovine ketosis c. Pregnancy toxaemia.

Notes: 1. Choice of glucocorticoid may vary with the condition.
2. Dosage of medication varies with desired effect, agent used, and also duration of therapy.
3. In many condition, glucocorticoid is used as an adjunctive therapy.

IM sites in animals in shock is slow, glucocorticoids should be given IV. In shock, steroids diminish blood stasis and promote tissue perfusion (the use of glucocorticoids in septic or haemorrhagic shock is controversial despite the recognition of potential benefits). Hydrocortisone may also be injected into inflamed joints resulting from diseases such as gout. Although not preferred, hydrocortisone is used occasionally for systemic anti-inflammatory action.

Hydrocortisone soluble salts (e.g. butyrate or acetate) are used topically for a large variety of dermatological conditions (Table 51.6). It benefits by virtue of its anti-inflammatory, immunosuppressive, vasoconstrictor and anti-proliferative (e.g. scaling lesions) actions. Hydrocortisone creams and ointments are available without prescription in strengths ranging from 0.05% to 2.5% (depending on local regulations) with stronger for available by prescription only.

Dose

For adrenocortical hormone insufficiency

Dogs : 0.5 mg/kg, PO, 2 times daily.

For shock/adjunctive therapy of various forms of shock

Dogs & cats : 50 mg/kg, IV (Hydrocortisone sodi succinate). Dose may be repeated after 3 6 hours, if required.

For anti-inflammatory activity

Dogs : 5 mg/kg, PO, 2 times daily.

Dogs : 5 mg/kg, IV or IM, once daily.

Cats : 5 mg/kg, PO, IV or IM, 2 times daily.

Table 51.4: List of important adverse effects associated with glucocorticoids.

	System	Effect
1.	Endocrine	a. Adrenal atrophy b. Iatrogenic Cushing's syndrome c. Suppression of hypothalamic-pituitary-adrenal (HPA) axis d. Decreased plasma thyroxine (T4)/ triiodothyronine (T3) and thyroid stimulating hormone (TSH). e. Enhanced parathormone secretion f. Growth retardation
2.	Dermatological	a. Acne/Comedones b. Cutaneous atrophy c. Excess bruising d. Hirsutism e. Telengiectasis f. Delayed wound and surgical incisions healing
3.	Respiratory	a. Pulmonary thromboembolism
4.	Gastrointestinal	a. Gastric/Colonic ulceration b. Gastric haemorrhage c. Thinning of gastrointestinal mucosa d. Reduced mucus production e. Gastric/Intestinal perforation f. Reduced gastrointestinal motility g. Pancreatitis h. Hepatocellular swelling/ Hepatomegaly
5.	Nervous	a. Behaviour changesb. Peripheral neuropathies
6.	Ophthalmological	a. Glaucoma b. Cataracts
7.	Cardiovascular	a. Hypertension b. Endocarditis
8.	Musculo-skeletal	a. Osteoporosis b. Vertebral compression c. Enhanced bone fractures d. Myopathy/Muscle weakness e. Muscle wasting f. Laminitis
9.	Reproduction/ lactation	a. Infertility b. Abortion c. Birth defects/Teratogenicity d. Retained placenta e. Transient fall in milk yield
10.	Renal/Electrolyte and water	a. Polyuria and compensatory polydipsia b. Glycosuria c. Sodium and water retention, oedema (with agent having mineralocorticoid activity) d. Hypokalaemia e. Metabolic alkalosis
11.	Immunological	a. Immunosuppression b. Progression of concurrent infection/Septicaemia
12.	Metabolic	a. Glucose intolerance b. Insulin resistance c. Overt diabetes mellitus d. Hyperlipidaemia

Notes: 1. Most adverse effects occur with prolonged glucocorticoid therapy.
2. All adverse effects may not be observed in a single animal.

For adjunctive treatment of photosensitization reactions

Cats : 100 – 600 mg (total) in 1 L of 10% dextrose saline, IV or SC.

Cortisone

Cortisone is a synthetic 17-hydroxy-11-dehydrocorticosterone, a replica of natural corticosteroid produced by the adrenal cortex. It is inactive as such hence requires an activating transformation (11β-hydroxylation) in liver to yield hydrocortisone. It is useful after oral and IM route for generalised types of systemic corticosteroid action, but is ineffective topically. Cortisone is only occasionally used now.

Dose

Cortisone acetate

Dogs & cats : 1 mg/kg, PO or IM, once daily.

2. Intermediate Acting Glucocorticoids

Prednisolone

Prednisolone (Deltahydrocortisone) is a synthetic glucocorticoid that is prepared by insertion of a second double bond between C1 and C2 in the ring A of hydrocortisone. This dehydrogenated analogue of hydrocortisone is slightly soluble in water and alcohol. The acetate ester of prednisolone is practically insoluble in water, while its sodium succinate ester is highly water-soluble.

Pharmacological effects

The mechanism of action and pharmacological effects of prednisolone are similar to those of hydrocortisone. However, it is more selective glucocorticoid with less retention of electrolytes and fluids than hydrocortisone (Table 51.5). Further, prednisolone is about 4 to 5 times more potent than hydrocortisone and is also less susceptible to metabolic inactivation (plasma t½ dogs = 1-3 hours). Prednisolone has an intermediate duration of action (12 to 36 hours) and is therefore ideal for alternate-day administration.

Side effects/Adverse effects

Side/Adverse effects of prednisolone are similar to hydrocortisone. Prednisolone causes less pituitary-adrenal suppression when alternate day treatment is given. High doses of prednisolone carry risk of laminitis in horses.

Clinical uses

Prednisolone is used in veterinary medicine for various inflammatory and allergic disorders and autoimmune diseases. Immune mediated diseases that may show response to high dose of prednisolone include immune-mediated haemolytic anaemia, thrombocytopenia, myasthenia gravis, arthritides, systemic lupus erythematous and pemphigus variants. It is used also for the treatment of adrenocortical insufficiency and in cancer therapy. Occasionally it is used for adjunctive therapy of endotoxaemic or septic shock. Prednisolone is used topically in treating dermatoses without evidence of systemic effects.

Dose

For inflammatory disorders of respiratory, liver or GI tracts

Dogs & cats : 0.5 – 2 mg/kg, PO once daily. For prolonged treatment, dose should be gradually reduced to lowest effective level and given on alternate days in morning for dogs and in evening for cats.

For dermatological or immune mediated disorders

Dogs : 1 – 2 mg/kg, PO, 2 times daily for 7 to 10 days, then to minimum effective alternate day dosage.

Cats : 2 – 4 mg/kg, PO, daily in divided doses. Dose may be tapered to alternate day low-dose therapy soon.

For adjunctive therapy of endotoxaemic/septic shock

Dogs : 5 – 10 mg/kg, IV of prednisolone sodium succinate. Dose may be repeated at 1, 3, 6 or 10 hours.

For chronic or subacute adrenal insufficiency

Dogs : 0.2 – 0.4 mg/kg, PO, once daily.

For hypoadrenal crisis/Acute adrenal insufficiency

Dogs : 4 – 20 mg/kg, IV over 2 to 4 minutes of prednisolone sodium succinate. May be repeated in 2 to 6 hours.

For various glucocorticoid effects

Cattle, horses, and swine : 0.2 – 1 mg/kg of prednisolone sodium succinate, IV or IM.

Prednisone

Prednisone (Deltacortisone) is a synthetic dehydrogenated analogue of corticosterone that is obtained by insertion of a second double bond between C1 and C2 in the ring A of cortisone. Prednisone is a prodrug, which is rapidly metabolised by the liver *in vivo* to prednisolone. Except of patients in frank hepatic failure, prednisone has about the same effectiveness as prednisolone. Because it is converted to the active compound prednisolone by the liver; prednisone is not considered suitable in patients with liver disease. It is usually taken orally but can be delivered by intramuscular, subcutaneous or intravenous injections. Prednisone is not suitable for topical application.

Dose

For various glucocorticoid effects

Dogs : 0.5 – 1 mg/kg, PO, IM, IV or SC, 1 to 2 times daily initially, followed by tapering to the lowest level, every other day.

Horses : 1 mg/kg, PO, 2 times daily for 1 week, then decrease by 25% each week.

For hypoadrenocorticism (acute or chronic)

Dogs : 0.2 – 0.4 mg/kg, PO, once daily.

Methylprednisolone

Methylprednisolone (6-α-Methylprednisolone) is a synthetic glucocorticoid, a slight chemical variant of prednisolone. It is slightly more potent and more selective in its glucocorticoid effects but with less retention of electrolytes (Table 51.5). Its pharmacological effects and clinical uses are similar to those of prednisolone.

Methylprednisolone has an intermediate duration of action and is suitable for alternate day therapy. Methylprednisolone possesses potent lipid antioxidant activity that has been found beneficial in the treatment of central nervous system trauma. Pulse therapy (parenteral administration of supra-pharmacological doses of short intermediate acting glucocorticoids for short period of time) with methylprednisolone has been tried in dogs to induce remission or control of autoimmune skin diseases and other conditions. The pulse therapy has been associated with some severe adverse reactions (e.g. arthralgia, facial swelling and seizures), therefore it should be reserved for patients those are intolerant to the standard protocol. Occasionally, repository (depot) methylprednisolone is used also in animals but should be reserved for patients in which administration of oral medication is not possible or for disorders for which only 2 or 3 treatments are required. Depot preparation is not suitable for alternate day therapy due to suppression of HPA axis.

Dose

For various inflammatory disorders

Methylprednisolone

Dogs & cats : 0.25 – 1 mg/kg, PO, 2 to 3 times daily for 5 to 10 days. After clinical signs are suppressed, dose may be given once daily for next about 1 week, followed by tapering to lowest effective alternate dosage.

Horses : 0.5 mg/kg, PO.

Methylprednisolone acetate (Depot preparation)

Dogs : 1 mg/kg, SC or IM. Dose may be repeated in 4 weeks.

Cats : 2 – 5 mg/kg, SC or IM. Dose may be repeated after 14 days.

: 10 – 40 mg (total), intralesional.

: 10 – 20 mg per joint, intra-articular.

Horses : 200 mg (total), IM. Dose may be repeated, as necessary.

Pulse therapy

For immunosuppressive action/Autoimmune diseases

Methylprednisolone sodium succinate

Dogs : 11 mg/kg in 250 ml of 5% dextrose (D5W), IV infusion over 1 hour, once daily for 3 days. After 3 days, oral administration of prednisone maintenance (1.1 mg/kg, once daily or every alternate day) should be started.

For CNS trauma/Spinal cord injury

Methylprednisolone sodium succinate

Dogs & cats : 30 mg/kg, IV. After 2 hours, drug is given at 15 mg/kg, IV. This is followed by 10 mg/kg, IV or SC, 4 times daily for 24-36 hours. Drug should be withdrawn gradually over next 7 days.

Triamcinolone

Triamcinolone is a synthetic glucocorticoid that is considered as a prednisolone derivative. It is equivalent to the prednisolone in systemic effects but lacks electrolyte-retaining activity of the group. It may even provoke diuresis and slight sodium loss in beginning. Triamcinolone is used to treat several different conditions such as eczema, psoriasis, arthritis, allergies, ulcerative colitis, uveitis

Table 51.5: Comparison of glucocorticoid and mineralocorticoid activities of important corticosteroids.

Corticosteroid	Duration of action	Anti-inflammatory potency (Glucocorticoid activity)	Sodium-retaining potency (Mineralocorticoid activity
Glucocorticoids			
1. Hydrocortisone	Short	1	1
2. Cortisone	Short	0.7	0.7
3. Prednisone	Intermediate	4	0.7
4. Prednisolone	Intermediate	4 – 5	0.7
5. Methyl prednisolone	Intermediate	5	0.5
6. Triamcinolone	Intermediate	3	0
7. Isoflupredone	Intermediate	10	0
8. Paramethasone	Long	10	0
9. Dexamethasone	Long	25 – 30	0
10. Betamethasone	Long	25 – 30	0
11. Flumetasone	Long	30	0
Mineralocortioids			
1. Fludrocortisone	Short	10	125
2. Aldosterone	Short	0.3	3000
3. Desoxycorticosterone	Long	0	100

ocular inflammation and in prevention of asthma attacks. Triamcinolone may cause hypoproteinaemia, cutaneous erythema, dizziness and sleepiness as side effects. Different triamcinolone salts are available in market, including acetonide, benetonide, furetonide, hexacetonide and diacetate. Triamcinolone acetonide is a more potent type of triamcinolone, which is not used for alternate day therapy due to enhanced suppression of HPA axis.

Dose

For various glucocorticoid effects

Dogs & cats : 0.11 – 0.22 mg/kg, PO, once daily for 7 days.
: 0.11 – 0.22 mg/kg, IM or SC. Effect generally persists for 7-15 days. If symptoms recur, parenteral dose may be repeated or oral therapy may be instituted.
: 1 – 2 mg per lesion, intralesionally at 0.5 to 2.5 cm intervals; not to be administered more than 0.6 mg at any one site and 6 mg total dose.
Dogs : 5 mg per joint, intra-articularly.
Cats : 1 – 3 mg per joint, intra-articularly.
Cattle : 0.02 – 0.04 mg/kg, IM.
: 6 – 18 mg (total), intra-articularly.
Horses : 0.1 – 0.2 mg/kg, IM or SC.
: 0.01 – 0.02 mg/kg, PO, 2 times daily.
: 6–18 mg (total), intra-articularly or intrasynovially; may be repeated after 3 to 4 days.

Isoflupredone

Isoflupredone (9-Fluoroprednisolone) is a synthetic fluorinated prednisolone derivative. It is more potent than prednisolone but possesses very slight or no mineralocorticoid activity. Isoflupredone acetate suspension is used for injection. Currently its use has declined in veterinary medicine.

Dose

Cattle & horses : 5 – 20 mg (total), IM, once daily.
: 5 – 20 mg, intrasynovially.

Deflazacort

Deflazacort is a glucocorticoid derived from prednisolone. Its mechanism of action and pharmacological effects are similar to those of prednisolone with 6 mg of deflazacort having approximately the same anti-inflammatory potency as 5 mg prednisolone or prednisone. Deflazacort can be used to treat a wide range of allergic and inflammatory conditions, including severe asthma and rheumatoid arthritis. It is available in tablet form for oral use.

3. Long-Acting Glucocorticoids

Dexamethasone

Dexamethasone (9α-Fluoro-16α-methyl prednisolone) is a highly potent and long acting synthetic glucocorticoid. Dexamethasone occurs as an odourless, white crystalline powder that is practically insoluble in water and sparingly soluble in alcohol. Commercially, dexamethasone is available in various salt forms like dexamethasone-acetate, -sodium phosphate, -isonicotinate and -trioxaundecanoate. Dexamethasone is available commercially in preparations of tablets, suspension for injection, powder and ophthalmic suspension.

Pharmacological effects

The mechanism of action and pharmacological effects of dexamethasone is similar to those of hydrocortisone. However, dexamethasone is about 20 to 30 times more potent than the naturally occurring hormone hydrocortisone and 4 to 5 times more potent than prednisone. It has virtually no mineralocorticoid activity. Dexamethasone also possesses some lipid antioxidant activity. Dexamethasone (also betamethasone) is preferred in cerebral oedema and other states in which fluid retention must be avoided.

Pharmacokinetics

Dexamethasone is a long-acting glucocorticoid. Its plasma half-life in dogs is about 2 hours but biological half-life is more than 48 hours.

Side effects/Adverse effects

Side/adverse effects of dexamethasone are about the same as produced with hydrocortisone but fluid retention and hypertension are not a problem. Dexamethasone causes marked suppression of HPA axis because of its prolonged action and high glucocorticoid potency.

Contraindications and precautions

Unnecessary prolonged therapy with dexamethasone and other long acting glucocorticoids should be avoided in order to minimise the possibility of adrenal insufficiency/adrenal atrophy. Like other glucocorticoids, dexamethasone should be used with extreme caution in pregnant animals because it may cause abortion and foetal abnormalities. The use of dexamethasone and other glucocorticoids to induce parturition is associated with an increased incidence of retained placenta in cattle, although subsequent fertility may not be affected.

Clinical uses

Dexamethasone is used widely in veterinary medicine for inflammatory and allergic conditions. Early administration of large doses of IV dexamethasone may be of benefit in acute circulatory failure or shock irrespective of the cause when given along with IV fluid therapy. Dexamethasone is also used for induction of parturition in cattle and sheep in late pregnancy. Administration of a single injection of dexamethasone to a cow in late pregnancy is followed by parturition within 72 hours. Dexamethasone and some other glucocorticoids are frequently used in the treatment of ketosis in cattle and goats and also for pregnancy toxaemia in sheep and goats. Intravenous dexamethasone therapy is suitable for treatment of acute primary hypoadrenocorticism until oral therapy can be tolerated.

Dexamethasone is occasionally used in low and high doses for the differential diagnosis of various forms of hyperadrenocorticism and to test the integrity of HPA axis. At low doses (low dose dexamethasone suppression test), dexamethasone suppresses ACTH secretion in normal animals with subsequent fall in hydrocortisone level. However in patients with pituitary dependent tumours, hyperadrenocorticism or adrenocortical tumour, there is continuous secretion of hydrocortisone and hence suppression test will not occur. The high dose suppression test might help distinguish between pituitary dependent disease and adrenal tumour. High dose of dexamethasone may cause the overactive pituitary gland to decrease ACTH secretion, but adrenal tumour will not be inhibited. Dexamethasone is generally used for these diagnostic purposes because it does not cross react with test reagents.

Dose

For therapeutic purposes

For various inflammatory conditions

Dogs & cats : 0.05 – 0.125 mg/kg, PO, IM, SC, or slow IV, once daily.

Horses : 0.05–0.2 mg/kg, IM, PO, or slow IV, once daily.

Cattle : 0.06 – 0.1 mg/kg, IM, once daily.

Swine : 1 – 10 mg (total), IM or slow IV.

For adjunctive therapy of shock

Dogs & cats : 4 – 6 mg/kg of dexamethasone sodium phosphate, slow IV, followed in 6 to 8 hours by 0.5 – 1 mg/kg, IM or SC. May be repeated after 8-12 hours.

For induction of parturition

Cattle : 20 – 30 mg (total), IM, as a single dose given within 2 weeks of normal term.

Sheep : 8 – 16 mg (total), IM as a single dose.

For termination of pregnancy/abortion

Cattle : 25 mg (total), IM with prostaglandin $F_{2\alpha}$ (25 mg total) after 150 days of parturition.

For bovine ketosis

Cattle : 5 – 20 mg (total), IM or slow IV, daily.

For diagnostic purposes

Low dose dexamethasone test

Dogs : 0.01 – 0.015 mg/kg, slow IV. Samples should be collected for ACTH estimation immediately before and at 4 and 8 hours after dexamethasone administration.

High dose dexamethasone test

Dogs & cats : 0.1 mg/kg, slow IV. Blood samples should be collected for ACTH estimation immediately before and at 4 and 8 hours after dexamethasone administration.

Note: Dexamethasone 1 mg = dexamethasone acetate 1.1 mg = dexamethasone isonicotinate and dexamethasone sodium phosphate 1.3 mg = dexamethasone trioxa-undecanoate 1.4 mg.

Betamethasone

Betamethasone (Flubenisolone) is a highly potent synthetic glucocorticoid that is structurally and pharmacologically similar to dexamethasone. It differs from dexamethasone only in the orientation of a side chain. Like dexamethasone, its glucocorticoid potency is about 30 times more than that of hydrocortisone and has virtually no mineralocorticoid activity. It has long-duration of action (biological half-life approximately 48 hours). Betamethasone is available as the base and as the dipropionate, acetate and sodium phosphate salts. The base is used for oral dosage forms. The sodium phosphate and acetate salts are used in injectable preparations, while dipropionate salt is used in topical formulations and in combinations with sodium phosphate salt in a veterinary approved injectable preparation. Insoluble ester provides 4-5 weeks duration action.

Similar to dexamethasone, betamethasone is used for the treatment of various inflammatory and allergic conditions, shock, ketosis and for induction of parturition in cattle. Unlike dexamethasone and other glucocorticoids, betamethasone is reported to cause decrease sperm output and semen volume in dogs.

Dose

For inflammatory disorders

Dogs & cats : 0.025 mg/kg, PO, once daily. Dose may be adjusted for individual animal.

All species : 0.04 – 0.08 mg/kg, IM or slow IV.

For induction of parturition

Cattle : 20 – 30 mg (total), IM. Dose may be repeated after 3 days, if required.

For ketosis

Cattle : 0.04 – 0.08 mg/kg, IM, or IV.

Flumetasone

Flumetasone (Flumethasone, 6α,9α-difluoro-16α-methyl prednisolone) is probably the most potent glucocorticoid. It possesses strong anti-inflammatory effects with little or no mineralocorticoid activity. It is used in various inflammatory and allergic disorders, shock and ketosis. It is useful for musculoskeletal conditions due to inflammation where permanent changes do not occur and for certain acute and chronic dermatoses.

Dose

For various inflammatory conditions/Acute and chronic dermatoses

Dogs : 0.06 – 0.25 mg (total), slow IV, IM, SC, or PO, once daily.
: 0.16 – 1 mg (total), intra-articularly.
: 0.125 – 1 mg (total), intralesionally.
Cats : 0.03 – 0.125 mg (total), slow IV, IM, SC, or PO.
Horses & cattle: 1.25 – 2.5 mg (total), slow IV, IM, or intra-articularly.

Parametasone

Parametasone is a synthetic long-acting glucocorticoid, which has intermediate properties between prednisolone and dexamethasone. It is recommended for the treatment of all conditions in which corticosteroid therapy is indicated except adrenal-deficiency states for which its lack of sodium-retaining properties makes it less suitable than hydrocortisone.

Glucocorticoids Used By Topical and/or Inhalation Route

A large number of glucocorticoids are used topically on skin or mucous membrane for treatment of many types of inflammatory dermatoses. Some agents have found usage exclusively in the eye for ophthalmic conditions responsive to glucocorticoids. In a similar fashion, some glucocorticoids are used primarily by nasal or oral inhalation for prevention and treatment of various disorders of upper respiratory tract including allergic and non-allergic rhinitis, sinusitis and asthma. The list of agents marketed primarily for dermatological, ophthalmological and pulmonary conditions is given in Table 51.6.

Beclometasone

Beclometasone (Beclomethasone) dipropionate is a synthetic halogenated glucocorticoid with high topical and low systemic activity. Its low systemic concentrations are due to its poor absorption and/or marked first pass metabolism. Beclomethasone is used as an inhaled steroid for treatment of rhinitis, sinusitis and prevention of asthma. Beclomethasone and other inhaled glucocorticoid reduce bronchial hyperactivity, increase peak expiratory flow rate, prevent episodes of acute asthma and reduce need for frequent beta-adrenoceptor agonist inhalation. However, they have no role during an attack or in status asthmaticus. Peak effect of beclometasone is seen after 4 to 7 days and benefit persists for a week after discontinuation. As a cream or ointment, beclometasone is used to treat severe inflammatory skin disorders (e.g. eczema) unresponsive to less potent steroids. It is also licensed to treat ulcerative colitis in conjunction with doses of 5-aminosalicylates.

When given by nasal inhalation in the form of an aqueous or aerosolized suspension, the drug is deposited primarily in the nasal passages. It is metabolized and cleared rapidly from the systemic circulation by metabolism mediated via esterase enzymes which are found in most tissues. Beclometasone is well tolerated. Occasionally it may cause a cough upon inhalation. Other side effects observed in human patients may rarely include unpleasant taste, hoarseness or nasal congestion, pain or headache and visual changes. Allergic reactions rarely may occur. Nasal corticosteroids may be associated with central serous retinopathy.

Dose

Horses : 1 – 3 µg (microgram)/kg (12 – 15 puffs), inhalation using metered dose inhalant system, 2 times daily.

Table 51.6: List of important corticosteroids used by topical or inhalation route.

Drug	Route of Administration/ Application	Important clinical use
Alclometasone dipropionate	Topical use on skin	Skin disorders like allergic dermatitis, atopic dermatitis, eczema, psoriasis and itching.
Amcinonide (Cyclocort)	Topical use on skin	Itching, redness, swelling associated with atopic dermatitis and allergic contact dermatitis.
Beclometasone (Beclomethasone) dipropionate	Inhalation	Rhinitis (e.g. hayfever) and sinusitis; prophylaxis of asthma.
	Topical use on skin	Inflammatory skin disorders (e.g. eczema) unresponsive to less potent steroids.
Betamethasone dipropionate	Topical use on skin	Skin disorders like itching, eczema and psoriasis.
Budesonide	Inhalation	Asthma and non-infectious rhinitis.
Ciclesonide	Inhalation	Maintenance treatment in persistent asthma and hayfever.
Clobetasone	Topical use on skin	Eczema and other forms of dermatitis.
	Topical use in eye	Ocular inflammation and pain.
Clobetasol propionate	Topical use on skin	Skin disorders including contact dermatitis, eczema, psoriasis and autoimmune skin conditions (alopecia areata and vitiligo).
Clocortolone pivalate	Topical on skin	Skin disorders including swelling, itching, redness associated with eczema, dermatitis, allergies and rash.
Desonide	Topical on skin	Skin disorders like atopic dermatitis, seborrheic dermatitis, psoriasis, eczema and contact dermatitis.

Contd table .....

Drug	Route of Administration/ Application	Important clinical use
Desoximetasone	Topical on skin	Various skin disorders having swelling, itching and rashes.
Dexamethasone sodium phosphate	Topical on skin Topical in eye	Various skin disorders having swelling, itching and rashes. To treat or prevent many eye conditions including swelling, itching and pain.
Diflorasone diacetate	Topical on skin	Various skin disorders having inflammation and itching.
Difluprednate	Topical in eye	Post-operative ocular inflammation and pain.
Fluclorolone acetonide	Topical use on skin	Various skin disorders having inflammation and irritation.
Fludroxycortide (Flurandrenolone or flurandrenolide)	Topical use on skin	Various skin disorders having inflammation and irritation.
Flumetasone Flunisolide	Topical use on skin Inhalation	Various skin disorders including otitis externa and otomycosis. Allergic rhinitis and asthma.
Fluocinolone acetonide	Topical use on skin	To reduce skin inflammation and relieve itching.
Fluocinonide	Topical use on skin	Skin disorders such as eczema itching, redness, dryness, crusting, scaling, inflammation and pain.
Fluocortolone	Topical use on skin	Skin disorders such as eczema and psoriasis.
Fluorometholone	Topical use in eye	Inflammatory conditions of the palpebral and bulbar conjunctiva, cornea and anterior segment of eye.
Fluperolone acetate	Topical use on skin	For inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses.
	Topical use in eye	Treatment of chronic non-infectious uveitis.
Fluprednidene	Topical use on skin	Corticosteroid-responsive dermatoses.
Flurandrenolide	Topical use on skin	Inflammation and itching associated with skin conditions such as allergic reactions, eczema and psoriasis.
Formocortal (Fluoroformylone)	Topical use in eye	Inflammatory disorders of eye.
Halcinonide	Topical use on skin	Inflammation and itching associated with allergic reactions, eczema and psoriasis.
Hydrocortisone acetate, butyrate and valerate	Topical use on skin	Irritations, itching and rashes caused by eczema, insect bites, poison ivy, soaps, detergents, etc. Applied locally to relieve pain and swelling due to arthritis, bursitis, etc.
Loteprednol etabonate	Topical use in eye	Inflammatory disorders of eye due to allergies as well as chronic forms of keratitis and vernal keratoconjunctivitis.
Medrysone	Topical use in eye	Allergic conjunctivitis, vernal conjunctivitis, episcleritis and epinephrine sensitivity.
Methylprednisolone acetate	Topical on skin	Several skin disorders including localized inflammatory and allergic lesions.
Mometasone	Topical on skin	Inflammatory skin disorders such as eczema, psoriasis, allergic dermatitis, atopic dermatitis, contact dermatitis and otitis externa.
	Inhalation	Allergic rhinitis such as hay fever and in asthma.
Prednicarbate	Topical on skin	Inflammation and itching associated with allergic reactions, eczema and psoriasis.
Prednisolone acetate and sodium phosphate	Topical use in eye	Allergic and inflammatory conditions of conjunctiva, cornea and anterior segment of the globe. Treatment of corneal, conjunctival and scleral injuries from chemical, radiation or thermal burns or penetration of foreign bodies.
Rimexolone	Topical use in eye	Postoperative inflammation following ocular surgery and in the treatment of anterior uveitis.
Tixocortol pivalate	Topical on skin	Eczema and other local inflammations.
Triamcinolone acetonide	Topical on skin	Swelling, itching and redness associated with several skin conditions such as eczema, dermatitis, allergies and rash.

Budesonide

Budesonide is a non-halogenated glucocorticoid with high topical : systemic activity ratio. It is claimed to be better than beclometasone as inhaled steroid. Budesonide is marketed mainly for the treatment of asthma and non-infectious rhinitis (including hay fever and other allergies). Budesonide in comparison with prednisolone has been associated with fewer bone density losses and, unlike other corticosteroids, has little influence on the hypothalamic-pituitary-adrenal axis. Nasal irritation, hoarseness, dry mouth rash, cough, gastric upsets are some of the side/ adverse effects observed in human patients.

Mometasone

Mometasone furoate is a synthetic glucocorticoid steroid used topically on the skin to relieve itching and inflammation of eczema, dermatitis, allergy and other skin rashes. It is also used by inhalation for various respiratory disorders including allergic rhinitis and asthma for patients unresponsive to less potent corticosteroids. It is marketed as a cream, ointment or lotion for skin conditions, a nasal spray for upper respiratory conditions and a dry powder inhaler (DPI) for lower respiratory conditions. It is also available as a veterinary drug in an otic (ears) form for treatment of otitis externa.

2. MINERALOCORTICOIDS

Fludrocortisone

Fludrocortisone (Fluohydrocortisone, fluohydrisone, 9α-fluorocortisol) is a synthetic halogenated form of hydrocortisone, which possesses a significant mineralocorticoid and moderate glucocorticoid activities. It is insoluble in water and slightly soluble in alcohol. It mainly occurs as acetate salt that is hydroscopic white to pale yellow powder or crystals.

Pharmacological effects

Fludrocortisone acetate is a potent corticosteroid. It is considered to be about 10 times as potent a glucocorticoid agent as hydrocortisone and is about 125 times more potent a mineralocorticoid than the hydrocortisone. It acts like endogenous aldosterone at the renal distal tubule to increase absorption of sodium. It also enhances excretion of potassium and hydrogen ion.

Pharmacokinetics

Fludrocortisone is well absorbed after oral administration. It has a short plasma half-life of about 3.5 hours, but its biological action persists for about 18-36 hours. Fludrocortisone acetate is administered orally at 24 to 48 hour intervals. Significant amount of fludrocortisone is excreted in milk.

Side effects/Adverse effects

Side/adverse effects of fludrocortisone are generally as a result of excessive sodium and water retention. Adverse effects are mostly seen at high doses, which are characterised by oedema, hypertension and hypokalaemia. Since fludrocortisone also possesses significant glucocorticoid activity, it may cause adverse effects associated with glucocorticoids (see for detail, Hydrocortisone).

Treatment: High dosage of fludrocortisone may be treated symptomatically and require discontinuation of drug until symptoms and signs associated with overdosage have resolved. Then drug should be started at a lower dosage.

Contraindications and precautions

Fludrocortisone is a potent mineralocorticoid hence electrolytes should be aggressively monitored during the treatment and potassium may need to be supplemented, if required. As it is excreted in milk, young ones suckling the mother receiving fludrocortisone may be given milk replacer after colostrum is consumed.

Drug interactions

Concomitant administration of fludrocortisone with potassium-depleting diuretics or amphotericin B may develop hypokalaemia. A diuretic can cause a loss of sodium and may antagonise the effects of fludrocortisone. Fludrocortisone may increase the insulin requirement of diabetic patients.

Clinical uses

Fludrocortisone has high mineralocorticoid and glucocorticoid activities and is used orally hence it is the preferred drug for the treatment of hypoadrenocorticism (Addition's disease). It is also used as adjunctive therapy in hyperkalaemia. In humans, fludrocortisone is used also in salt losing form of congenital adrenal hyperplasia in patients with severe postural hypotension.

Dose

For hypoadrenocorticism

Dogs & cats : 0.015 – 0.02 mg/kg, PO, once daily. The dose may be increased during first 6 to 8 months of therapy and may be required twice daily in a few cases. Serum sodium and potassium concentrations should be monitored carefully every 1-2 weeks and dosage may be adjusted.

For adjunctive therapy of hyperkalaemia

Dogs : 0.1 – 1 mg (total), PO, daily.

Desoxycorticosterone

Desoxycorticosterone (Deoxycorticosterone, DOC) is a mineralocorticoid with almost no glucocorticoid activity. It is available as desoxycorticosterone pivalate (DOCP) and desoxycorticosterone acetate (DOCA). Desoxycorticosterone pivalate is a long-acting mineralocorticoid agent with 20 to 30 days duration of action after intramuscular injection. It increases the absorption of sodium at the renal distal tubule.

Desoxycorticosterone is indicated for the treatment of adrenocortical insufficiency in dogs. Addisonian patients those have failed to completely respond to fludrocortisone may respond to therapy with DOCP. As the drug lacks glucocorticoid effect, all animals with adrenocortical insufficiency treated with DOCP may require additional glucocorticoid during the periods of stress or acute illness. Adverse reactions with overdosage of DOCP include polyuria, polydipsia, hypernatraemia, hypertension, oedema, hyperkalaemia, cardiac enlargement and congestive heart failure.

Dose

Desoxycorticosterone pivalate

Dogs : 2.2 mg/kg, IM, every 25 days. Serum potassium and sodium levels should be monitored carefully and dosage may be adjusted as necessary.

Aldosterone

Aldosterone is the most potent mineralocorticoid. However, it is not used clinically because of low oral bioavailability and difficulties in regulating doses.

General Principles in Corticosteroid Therapy

Corticosteroids, especially glucocorticoids, are widely used in the treatment of numerous diseases (Table 51.3). They have potential to cause dramatic improvement in many severe diseases as well produce equally dramatic adverse effects, if not used properly (Table 51.4). Therefore, a specific course of therapy should be defined and as a matter of caution following general principles must be observed:

1. Dosage of a corticosteroid must be individualised because it varies with the potency, formulation, rate and route of administration of drug employed, nature and severity of condition treated, goals of therapy, and also patient's response to hormonal therapy.
2. Dosage of a corticosteroid is usually high for an acute and brief process and relatively low when prolonged medication is anticipated. In prolonged medication, it is important to seek the minimal effective dose to alleviate the condition.
3. Single dose, even excessive, is not harmful. Therefore, single dose of a corticosteroid can be used to tide over mortal crisis even when benefit is not certain. Starting dose can also be high in some severe illness.
4. Short courses, even at high dose, are not likely to be harmful in absence of contraindications. Prolonged therapy at high doses is potentially hazardous because it would cause systemic disturbances and may produce adrenal atrophy.
5. If an animal has been treated with intensive corticosteroid therapy for greater than 24 hours or therapy has continued for more than 2 weeks, the dose should be tapered over days (2 weeks or more), while increasing the interval between doses. This is because the recovery of HPA-axis occurs slowly over several weeks.
6. Abrupt termination of chronic corticosteroid therapy should always be avoided because it generally results in a hypoadrenocortical state.
7. The anti-inflammatory effects of corticosteroids is only palliative and as such do not cure any specific disease. Therefore corticosteroids should be used judiciously in the existing anti-inflammatory conditions and sometimes even be avoided.
8. Special caution must be exercised in the use of corticosteroids if an infectious disease including fungal or viral infection is present. The anti-inflammatory effect of corticosteroids may promote dissemination of infectious organisms throughout the body. In pre-existing infections, an appropriate antimicrobial drug should be administered at the same time, if a corticosteroid is used.
9. Corticosteroids should be administered locally whenever possible rather than systemically. Although some absorption may occur, but the low systemic corticoid level preserves the integrity of adrenal cortex while maintaining a high level of drug at the required site.

Drug Preparations and Routes of Administration

The corticosteroids used clinically are available in a number of topical and systemic preparations. Corticosteroid preparations are generally available either as free steroid alcohols or as esters. Some of the commonly used preparations of corticosteroid are mentioned below:

1. Oral preparations usually contain the free alcohol. Absorption of free steroid alcohol from the GI tract is generally quite rapid. Their duration of effects is almost the same as the biological half-life.
2. Parenteral preparations are either esters or free steroid alcohols.

a. Highly soluble esters (e.g. sodium succinate, hemisuccinate and phosphate) and free alcohols can be administered IV or IM. Intravenous route is used when high plasma or tissue concentration is required rapidly (e.g. shock or allergic reaction). The duration of action of free alcohol solutions or highly soluble esters approximates the biological half-life of the free corticosteroids.

b. Esters including acetate, dipropionate, isonicotinate, phenylpropionate, pivalate and trioxa-undecanoate are insoluble and are not used IV. These esters are indicated primarily for intralesional or intra-articular administration (repository or depot forms) and may also be administered SC or IM. The moderately insoluble esters (e.g. acetate, diacetate and tebulate) prolong absorption over days to weeks, whereas the poorly soluble esters (e.g. acetonide, hexacetate, pivalate and dipropionate) release corticosteroids over a period of weeks to months.

3. Several topical preparations are available for skin, ophthalmic and otic conditions. These corticosteroid preparations may be available as the free steroid alcohol or as an ester. Acetonide and valcrate esters bind to affected tissue, thereby enhancing the corticosteroid effect. These preparations provide high concentrations of a potent corticosteroid at the disease tissue site while minimising systemic effects.

II. Drugs used in Excess of Adrenocortical Hormones (Hyperadrenocorticism)

Hyperadrenocorticism can be managed by both surgical and non-surgical means. If an adrenal tumour is identified, then surgical removal may be a viable option. In the non-surgical management, several types of drug are used depending on the type and severity of disease and the kind of drug available. Non surgical treatment is the most often used treatment for most cases of canine Cushing's disease.

1. Inhibitors of Corticosteroid Biosynthesis

Mitotane

Mitotane (O,p'-DDD) is a cytotoxic agent that is structurally related to the organochlorine insecticides dichlorodiphenyl-trichloroethane (DDT). It occurs as a white crystalline powder and is practically insoluble in water. Mitotane is considered as the treatment of choice for hyperadrenocorticism in dogs.

Mechanism of action

Mitotane is a cytotoxic drug. It selectively destroys the zona fasciculata and zona reticularis of the adrenal cortex and decreases corticosteroid synthesis. Although the precise mechanism of action is not known, mitotane requires conversion to an intermediate metabolite O,p'-DDA to be active. The O,p'-DDA covalently binds to macromolecules in the cell mitochondria with

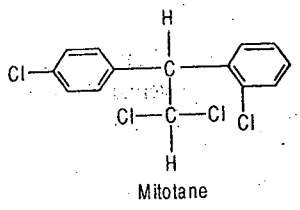


Fig. 51.6. Chemical structure of mitotane.

subsequent cell death and necrosis. The zona reticularis of adrenal cortex is most sensitive to the cytotoxic effect on mitotane, whereas the zona glomerulosa is least sensitive. Because zona glomerulosa is resistant to mitotane, the normal secretion of aldosterone is generally not disturbed.

In addition to cytotoxic effects, mitotane interferes with the steroid biosynthesis, primarily through inhibition of the 11β-hydroxylase and cholesterol side-cleavage enzyme. The exact mechanism by which mitotane impedes steroid hormones synthesis is not known, but it may be related to inhibition of glucose-6-phosphate dehydrogenase or low production of reduced triphosphopyridine in the adrenal cortical cells.

Pharmacological effects

Mitotane causes rapid reduction in the levels of adrenocortical steroids and their metabolites in blood and urine. In dogs with pituitary-dependent hyperadrenocorticism, mitotane causes severe and progressive necrosis of the zona fasciculata and zona reticularis in both normal and hyperplastic/neoplastic gland. Damage to liver, kidneys or bone marrow has not been reported with mitotane therapy.

Pharmacokinetics

The pharmacokinetic data on mitotane in domestic animals are limited. Mitotane is poorly absorbed after oral administration in empty stomach; giving drug with food can increase absorption. The availability of mitotane usually decreases as treatment proceeds, even if the drug is administered with food. In humans, approximately 40% of an oral dose of mitotane is absorbed with peak serum levels occurring about 3-5 hours after a single dose. It is distributed to virtually all tissues of the body. The drug is stored in adipose tissues but does not accumulate in adrenal glands.

Mitotane is metabolised in liver by microsomal enzyme system and is excreted as metabolites in the urine and bile. In humans (probably also dogs), the elimination of mitotane becomes more prolonged after repeated administration due to its initial deposition and then gradual liberation from adipose tissues. Mitotane has a very long plasma half-life in humans, with values ranging from 18-160 days. After discontinuation of therapy, plasma concentrations of mitotane can be measured for up to 6 to 9 weeks.

Side effects/Adverse effects

Most common adverse effects related to mitotane therapy in dogs include lethargy, ataxia, weakness, anorexia, vomiting or diarrhoea. These effects are attributable mainly to lowered hydrocortisone levels or a too rapid decrease of plasma hydrocortisone levels into the normal range. Neurological signs such as incoordination, circling, blindness, facial paralysis and seizures may also be observed. Hepatotoxicity (i.e. congestion, centrilobular atrophy and fatty degeneration) may also be seen in dogs given mitotane. Mitotane-induced hypoadrenocorticism may occur in animals. In about 5% of dogs treated with mitotane, fludrocortisone and long-term glucocorticoids may be needed as replacement therapy

Treatment: The therapy should be stopped temporarily and animals may be given glucocorticoids, if adverse effects are noted with mitotane. Overdosage of mitotane should be treated symptomatically and supportively.

Contraindications and precautions

Mitotane is contraindicated in patients known to be hypersensitive to it and in pre-existing liver disease. Dogs with pre-existing renal disease or diabetes mellitus should receive the drug with caution as mitotane rapidly changes insulin requirements during initial treatment period. Mitotane should be cautiously used in pregnant and lactating animals. Although it is not known if the drug is distributed into the milk, but it is suggested that puppies be given milk replacer after receiving colostrum, if the mother is receiving mitotane. All dogs receiving mitotane therapy should receive additional glucocorticoid supplementation, if undergoing a stress (e.g. surgery, illness and trauma).

Drug interactions

Any drug that stimulates hepatic microsomal enzyme system (e.g. phenobarbital) would enhance the metabolism of mitotane, resulting in diminished mitotane concentration and a diminished adrenocorticolytic effect. In addition to other concurrently administered drugs, mitotane may enhance its own biotransformation through inductions of microsomal enzymes synthesis. In diabetes mellitus, mitotane decreases requirement of insulin. Mitotane enhances CNS depressant effect of drugs, if used concurrently with CNS depressants. In dogs, spironolactone has been reported to block the action of mitotane.

Clinical uses

In veterinary medicine, mitotane is used primarily as adrenocorticolytic drug for the medical treatment of pituitary-dependent hyperadrenocorticism and also adrenal-dependent hyperadrenocorticism in dogs. It has also been used for the palliative treatment of adrenal carcinoma in humans and dogs. Although considerable care is required in its use, many cases have been successfully managed with this drug in the long-term use.

Therapy of hyperadrenocorticism with mitotane occurs in two phases - an induction phase and a maintenance phase. For induction, mitotane is given daily or every 12 hours until clinical signs of hyperadrenocorticism begin to resolve (e.g. animal's appetite or thirst returns to normal). Maintenance therapy is necessary for the rest of animal's life although dose and frequency may vary depending on the initial response to drug. If the animal has shown a rapid response to the induction therapy than it is considered more sensitive to mitotane and accordingly the maintenance dose is reduced. If the maintenance therapy is discontinued during the remaining life period of animal, the adrenal glands will once again become hyperplastic in response to continued ACTH secretion from the pituitary gland.

Dose

Dogs : 25 mg/kg, PO, 2 times daily (usually in food) until thirst/appetite returns to normal (usually 7 to 10 days). This is followed by 25-50 mg/kg, PO, every 1 to 2 weeks to prevent recurrence of clinical signs.

Ketoconazole

Ketoconazole is an imidazole derivative that is primarily used as an antifungal drug. It is used occasionally as an alternative to mitotane for the medical management of pituitary- and adrenal-dependent hyperadrenocorticism. Ketoconazole has a reversible inhibitory effect on glucocorticoid synthesis, but has negligible effects on mineralocorticoid production. It inhibits steroid synthesis directly by blocking several P-450 enzyme systems. In addition, ketoconazole appears to bind to glucocorticoid receptors. Although measurable reductions in hydrocortisone or testosterone synthesis occur at dosages used for antifungal therapy, higher dosages of the drug are generally required to reduce levels of hydrocortisone or testosterone to be clinically useful in the treatment of hyperadrenocorticism or possible prostatic carcinoma, respectively.

Ketoconazole is considered a safe drug, but occasionally hepatotoxicity may occur in some dogs. Other important adverse effects include anorexia, vomiting, diarrhoea and jaundice. Inhibition of hepatic cytochrome P-450 enzymes by ketoconazole decreases the metabolism of several drugs. A potentially life-threatening interaction between ketoconazole and non-sedating antihistamines has been demonstrated in man. The pharmacology of ketoconazole has been given in detail in Chapter 66.

Dose

For pituitary/adrenal-dependent hyperadrenocorticism

Dogs : 5 mg/kg, PO, 2 times daily for 7 days, increasing to 10 mg/kg twice daily for 7 to 14 days, then 15 mg/kg twice daily. Lower dosage is required initially to assess adverse reactions. The response is monitored using the ACTH stimulation test.

Metyrapone

Metyrapone is a drug used in the diagnosis of adrenal insufficiency and occasionally in the treatment of hyperadrenocorticism in cats. It impairs the biosynthesis of corticosteroids by inhibiting the enzyme 11β-hydroxylase, which converts 11-deoxyhydrocortisone to hydrocortisone. The reduction in hydrocortisone synthesis diminishes glucocorticoid feedback inhibition leading to increased ACTH release and an increased production of steroid intermediates proximal to the block (e.g. 11-deoxyhydrocortisone). In pituitary disease, production and subsequent urinary excretion of 11-deoxyhydrocortisone fails to increase in response to metyrapone administration. Thus metyrapone is used mainly to test the capacity of hypothalamic-pituitary mechanism to produce ACTH.

Dose

Treatment of hyperadrenocorticism

Cats : 65 mg/kg, PO, 2 to 3 times daily.

Aminoglutethimide

Aminoglutethimide is a toxic and an anti-steroid drug. It primarily inhibits the P-450-cholesterol side-chain cleavage enzyme ($P\text{-}450_{SCC}$), which converts cholesterol to pregnenolone. As a result, the production of all classes of steroid hormones is impaired. Aminoglutethimide also inhibits $P\text{-}450_{11}\beta$ and the enzyme aromatase, which convert androgens to oestrogens.

Aminoglutethimide has been used to decrease hypersecretion of hydrocortisone in human patients with Cushings's syndrome secondary to autonomous ectopic production in ACTH. Because of its action to inhibit aromatase, aminoglutethimide has also been recommended for the treatment of breast cancer in women. It has limited therapeutic application in veterinary medicine. The pharmacology of aminoglutethimide has been discussed also in Chapter 52.

Trilostane

Trilostane is a reversible inhibitor of 3-β-hydroxy-steroid dehydrogenase, which converts pregnenolone to progesterone. Trilostane reduces adrenocortical synthesis of both hydrocortisone and aldosterone and increases urinary excretion of 17-ketosteroids. The drug is occasionally used in human medicine in the treatment of Cushing's syndrome when more definitive therapy cannot be utilised. Trilostane is also occasionally recommended to treat both pituitary- and adrenal-dependent hyperadrenocorticism in dogs.

2. Inhibitors of Glucocorticoid Receptors

Mifepristone

Mifepristone is a synthetic steroid and a progesterone receptor antagonist, which at high doses also inhibit the glucocorticoid receptors. By blocking glucocorticoids action, it blocks feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis and thus secondarily enhances endogenous ACTH and hydrocorti-sone levels. It is primarily used as an abortifacient in human medicine (Chapter 52). Mifepristone has also been used to treat Cushing's syndrome in some cases.

3. Inhibitors of Aldosterone Receptors

Inhibitors of aldosterone receptor refer to drugs which antagonize the action of aldosterone at mineralocorticoid receptors. These drugs are largely used as diuretics for the management of chronic heart failure. Aldosterone receptor antagonists such as spironolactone, eplerenone, canrenone and potassium canrenoate have also found usage in the management of hyperaldosteronism, a medical condition where too much aldosterone is produced by the adrenal glands. The pharmacology of aldosterone receptor antagonists has been given in Chapter 39.

4. Inhibitors of ACTH Biosynthesis and Release

Pituitary dependent hyperadrenocorticism may be treated by drugs which decrease ACTH biosynthesis and release. These drugs include bromocriptine, pergolide (both dopamine receptor agonists), selegiline (monoamine oxidase inhibitor) and cyproheptadine (mixed histamine and serotonin receptors antagonist). The pharmacology and clinical uses of these drugs in pituitary dependent hyperadrenocorticism are described in Chapter 49.

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CHAPTER 52 GONADAL HORMONES

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Female sex hormones

The gonads in females are ovaries and in males are testes. The ovaries in females and testes in males produce steroidal hormones, which affect sexual behaviour, secondary sex characteristics, accessory sex organs and reproduction in general. The gonads of both female and male are controlled hormonally by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secreted by the anterior pituitary gland. The natural gonadal hormones of most concern include estradiol 17β and progesterone in the female and testosterone in the male. Their synthetic analogues have similar or antagonistic actions and are used therapeutically in replacement therapy and other reproductive disorders.

FEMALE GONADS

The female gonads or ovaries are the primary organs of reproduction in the female. The ovaries produce gametes and

hormones which regulate and integrate the functional activity of female reproductive system. Both gametogenesis and steroidogenesis are regulated primarily by the gonadotropins released by anterior pituitary under the influence of hypothalamic-releasing hormones (gonadotropin releasing hormone, GnRH). In addition, prostaglandins, relaxin, oxytocin, etc. also affect the reproductive system at different stages. Some of these hormones/mediators have been discussed in the appropriate chapters.

Ovarian Hormones/Female Sex Hormones

The ovaries produce two main types of steroid hormone, the oestrogens and the progestogens. The most important of oestrogens is the hormone estradiol 17β and the most important progestogen is the progesterone. The oestrogens promote mainly proliferation and growth of specific cells in body and are responsible for development of most secondary sexual characteristics of the female. The progestogens, on the other hand, are concerned almost entirely with final preparations of the uterus for pregnancy and mammary glands for lactation. In addition to the steroidal hormones, the ovaries are involved in the secretion of peptide hormones/factors such as oocyte mature inhibitor, inhibin, gonadocrinin and relaxin.

OESTROGENS

Oestrogens (Estrogens) are a group of compounds synthesised mainly by ovaries but also in fairly large amounts by placenta in pregnant animals and in small amounts by testes in males and by adrenal cortex in both sexes. Some other tissues such as liver, muscle and hair follicles can also convert steroid precursors into oestrogens. In ovaries, oestrogens are produced by the theca internal and granulose cells of the ovarian follicle under synergistic control of FSH and LH and possibly under local influence of intrafollicular factors such as inhibin and gonadocrinin. Oestrogens have immense importance in the oestrous cycle of animals and function as the primary female sex hormones.

Synthesis, Release, Transport and Fate of Oestrogens

Synthesis and release: The naturally occurring oestrogens are 18-carbon steroids, which are closely related to male sex hormones, adrenal steroids and bile acids and vitamin D. In females, synthesis of oestrogens starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol. Androstenedione, a substance of moderate androgenic activity, crosses the basal membrane into the surrounding granulosa cells, where it is converted to estradiol or estrone, either immediately or through testosterone by enzyme aromatase (Fig. 52.1). The synthesis of these oestrogens in the granulosa cells occurs during the follicular phase of ovarian cycle under the influence of pituitary hormone FSH. Estriol is an oxidative product derived from both estradiol and estrone, the conversion occurring mainly in the liver. Of various oestrogens, 17β-estradiol is the principal and most potent oestrogen secreted by ovary followed by estrone and estriol. The oestrogenic potency of 17β-estradiol is about 12 times that of estrone and 80 times that of estriol. It has been suggested the estrone is mainly a storage form of the oestrogenic hormones, as it remains in equilibrium with estradiol. Both estradiol and estrone are interconvertible.

Transport and fate: After synthesis in the ovary, a portion of oestrogen accumulates in the follicular fluid filling the antrum, but majority diffuse into the capillaries of thecal layer to be distributed into systemic circulation. Oestrogens are transported in blood bound mainly with plasma albumin and specific oestrogen binding globulins. The binding between oestrogens and plasma proteins is weak and these are rapidly released over a period of 30 minutes. Estradiol and estrone are converted to almost inactive estriol in the liver. In the liver also, various oestrogens are conjugated to form glucuronides and sulphates, which are excreted mainly in urine and also in bile. Estrone is also converted in body to estrone sulphate, a long-lived derivative. Estrone sulphate acts as a reservoir that can be converted as needed to the more active estradiol.

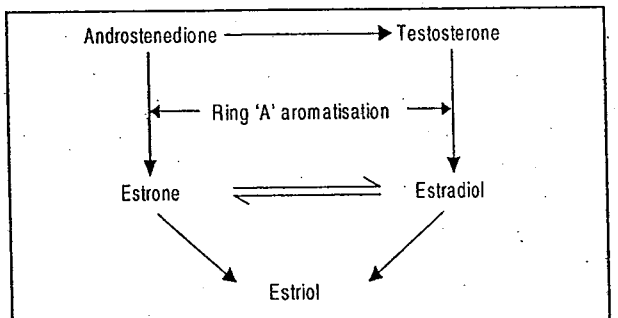


Fig. 52.1. Synthesis of oestrogens from androgenic precursors.

Mechanism of Action

Oestrogens mediate their effect through a group of receptors called the oestrogen/estrogen receptors (ER). Two types of oestrogen receptor have been identified. The first and well described type of receptor belongs to the superfamily of steroid receptors located intracellularly. A second and lesser known type of receptor, called oestrogen G protein-coupled receptor, has recently been identified on cell surface. The classical oestrogen effects are believed to be mediated through the nuclear oestrogen receptor and its action has been described below.

Like other steroid hormones, oestrogens diffuse across the cell membranes in target cells and bind with high affinity to specific intracellular oestrogen receptors. The activated oestrogen-receptor complex form dimers and interacts with nuclear chromatin to modulate the expression of many genes. This in turn results in regulation of specific protein synthesis that mediates a number of biological functions. One of the principal effects of oestrogens is the induction of synthesis of progesterone receptors in target tissue such as uterus, vagina, anterior pituitary and hypothalamus.

The intracellular oestrogen receptors occur primarily in the female reproductive tract (uterus and vagina), mammary glands, pituitary, hypothalamus, bone, liver, blood vessels and heart. The receptor has a molecular mass of approximately 66,000 Da. with a single hormone binding-site. The receptor has a distinct region or domain for hormone binding, a domain for DNA binding, and two other domains involved in transcriptional activation. Affinity for the receptors varies with the particular oestrogen. In the absence of hormone, oestrogen receptors are largely located in the cytosol. However, the hormone binding to the receptor triggers a number of events starting with migration of the receptor from the cytosol into the nucleus. Progesterone decreases oestrogen receptor expression in reproductive tract even in presence of continuously high plasma

oestrogen concentrations by interfering with the *de novo* synthesis of the receptors. Prolactin on the other hand, increases the number of oestrogen receptors in mammary gland and liver but has no effect in uterus.

Physiological Effects

The principal physiological role of oestrogens in general is the development and maintenance of female sex organs. Oestrogens are responsible for initiating behavioural signs of oestrus, preparing female reproductive tract for fertilisation, and developing secondary sex characteristics and secretory tissue of mammary gland.

Sex organs: Oestrogens are largely responsible for the development and maintenance of female sex organs. Oestrogens in general and 17β-estradiol in particular are feminine hormones responsible for the changes those take place at puberty i.e. growth of uterus, fallopian tube and vagina. Oestrogens are responsible for the marked cornification of vaginal epithelium in some species such as in bitches. In many domestic species, oestrogens alone or increasing blood levels of oestrogens in association with declining levels of progesterone induce behavioural changes. Oestrogens secreted by the developing follicle during the cycle induce pronounced vascularisation, increased blood flow and hyperaemia, enhanced uterine tone, salt and water retention, and oedema of uterus, vagina and vulva. After progesterone priming, oestrogens are responsible for induction of sexual receptivity to mating of the female. Oestrogens increase rhythmic contractions of fallopian tubes and uterus, cause cervical dilation and sensitise uterus to oxytocin. They induce a watery secretion from the cervix, favourable to sperm penetration. Oestrogens also promote induction of progesterone receptor, which enables cells to respond to progesterone during the second half of cycle. In absence of oestrogens, oestrus cycle disappears and reproductive tract atrophies. Therefore, oestrogens are usually termed as growth hormone of the genital tract, especially the myometrium, genital tract epithelium and vascular system.

Secondary sex characters: Oestrogens produced at puberty stimulate the growth of mammary glands. They induce development of ductal and stromal tissues of the mammary gland in all domestic species. Oestrogens make the voice shrill and hair and skin glossy in most species of animals. In birds, oestrogens form plumage and changes colour of beak. Oestrogens are responsible for the moulding body contours and shaping skeleton and also influence behaviour in females. They antagonise androgen receptors and are involved in feedback influence on the hypothalamic-pituitary-gonadal axis.

Metabolic effects: Oestrogens are anabolic, similar to but weaker than testosterone. They stimulate protein synthesis and enhance protein deposition in the body. This occurs probably from the growth-promoting effect of oestrogens on sexual organs, bones and some other tissues of body. Oestrogens inhibit growth of bones and favours ossification of epiphyseal lines. Oestrogens have also been observed to increase blood cholesterol and calcium in birds and some mammalian species. Oestrogens have epitheliotropic action, as they favour vascularity of capillaries in general and also health of skin. They also cause deposition of increased quantities of fat in the subcutaneous tissues; as a result, overall specific gravity of the female body contains more protein and less fat than that of the male body. Oestrogens also increase the coagulability of blood by increasing plasma concentrations of some clotting factors (e.g. Factors X and II) and platelet aggregation, and by decreasing antithrombin III concentration.

DISORDERS OF OESTROGENS

Deficiency of Oestrogens

As discussed earlier, oestrogens are required in appropriate concentrations in body for the normal functioning of reproduction in females. The quantity of oestrogens produced by ovaries must rise above a critical level for the normal rhythmical sexual cycles. Deficiency of oestrogens can result from ovarian hypoplasia, lack of ovaries, or genetically abnormal ovaries which secrete wrong hormones because of missing enzymes in the secretory cells. When ovaries are absent from birth or when they become non-functional before puberty, the usual secondary sexual characteristics do not appear and the sexual organs remain infantile. When ovaries undergo hypoplasia or become non-functional after puberty, the animal shows either an absence of oestrus cycle or only occasional oestrus. Prolonged ovarian cycles are frequently associated with failure of ovulation, presumably because of insufficient secretion of LH. Deficiency of oestrogens due to ovarian hypoplasia or gonadal dysgenesis or both may result in small flaccid uterus, flaccid cervix with open os, and small to very small ovaries with no follicles or corpora lutea. Infertility associated with ovarian hypoplasia and/or gonadal dysgenesis has been described in mares and may be seen in other species of animals.

Excess of Oestrogens

Excess secretion of oestrogens by the ovaries occurs rarely because hypersecretion of oestrogens automatically decreases the production of gonadotropins by pituitary, and this in turn limits the production of ovarian hormones. A rare granulosa cell tumour can develop in an ovary, which secretes large quantities of oestrogens. Such tumours, occasionally seen in mares, cause marked enlargement of one ovary and can secrete large quantities of oestrogens. Cystic ovary disease, commonly seen in dairy cattle and occasionally in dogs, cats, pigs and mares, is capable of steroidogenesis but its products vary from oestrogens to progesterone to androgens.

TREATMENT OF OESTROGEN DISORDERS

Disorders of oestrogens can have many patho-physiological and biological effects. Depending on the type of disorder, the oestrogen imbalance can be treated by using a wide variety of drugs possessing either oestrogenic activity or anti-oestrogenic activity.

Classification

Drugs used to treat oestrogen disorders have been broadly classified into two classes: Drugs used in deficiency of oestrogen and drugs used in excess of oestrogen.

I. Drugs used in deficiency of oestrogens / Oestrogenic drugs
   A. Steroidal oestrogens
      1. Natural steroidal oestrogens
         e.g. estradiol, estrone, equilin and equilenin.
      2. Semi-synthetic steroidal oestrogens
         e.g. estradiol cypionate, estradiol valerate, estradiol benzoate and estradiol propionate.
      3. Synthetic steroidal estradiols.
         e.g. ethinylestradiol, mestranol and quinestrol.

B. Non-steroidal oestrogens/Synthetic non-steroidal oestrogens.
e.g. diethylstilbestrol, hexoestrol, benzestrol, dienestrol, methallenoestrol, chlorotrianisene, fosfestrol and zeranol.

II. Drugs used in excess of oestrogens / Anti-oestrogenic drugs
A. Oestrogen receptor inhibitors
1. Mixed oestrogen receptor antagonist-agonists
e.g. clomifene, tamoxifen, toremifene and cyclofenil.
2. Selective oestrogen receptor antagonists
e.g. fulvestrant.
B. Aromatase Inhibitors/ Oestrogen synthesis inhibitors
1. Selective aromatase inhibitors
e.g. anastrozole, letrozole, exemestane and formestane.
2. Non-selective aromatase inhibitors
e.g. aminoglutethimide and testolactone.

I. DRUGS USED IN DEFICIENCY OF OESTROGENS / OESTROGENIC DRUGS

Oestrogens are endogenous steroid hormones which regulate and integrate the functional activity of female reproductive system. Natural oestrogens are inactive orally and have a short duration of action; therefore several non-steroidal synthetic compounds have been produced. These synthetic compounds possess oestrogenic activity similar to those of endogenous oestrogens and, therefore, are conventionally also called oestrogens (In pharmacology, oestrogen is used as a generic term for all compounds having oestrogenic activity).

Chemistry and Structure Activity Relationship

The naturally occurring oestrogens are all 18-carbon steroids containing a phenolic A ring (having a hydroxyl group at position 3) and a β-hydroxyl group or ketone in position 17 of ring D. Substitutions at different sites on the basic structure (Fig. 52.2) alter the activity of oestrogenic compounds.

The phenolic A ring is the principal structural feature required for selective high affinity binding to oestrogen receptors. Alkyl substitution on the phenolic A ring impairs binding with receptor. Ethinyl substitution at C-17 position increases oral potency of the compound by inhibiting its first-pass hepatic metabolism. Substitutions on ring C and D does not make much change in activity. Several non-steroidal compounds having activity similar to naturally occurring steroids have been synthesised. Some of these (e.g. diethylstilbestrol) assume a trans-configuration as depicted in Figure 52.3 and structurally resemble natural oestrogens. Diethylstilbestrol is as potent as estradiol, is orally active and has a longer half-life in the body.

A. Steroidal Oestrogens

1-2. Natural and Semi-synthetic Steroidal Oestrogens

Estradiol and Semi-synthetic esters of estradiol

Estradiol (Oestradiol) is a naturally occurring female sex hormone that is produced by ovaries (mainly) and placenta. Estradiol is poorly absorbed from the gut; therefore it has been chemically modified for administration by oral route. Semi-synthetic esters of

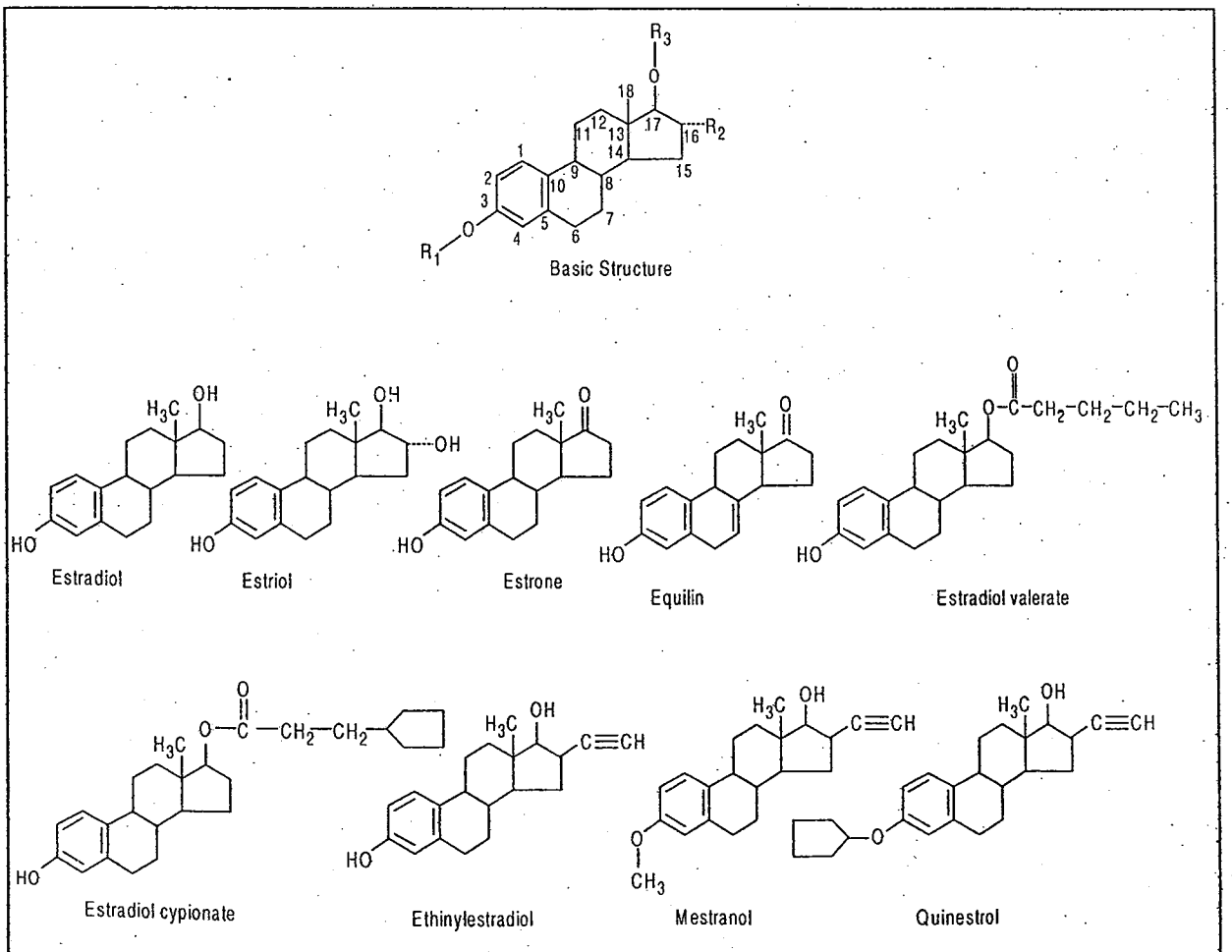


Fig. 52.2. Chemical structures of selected steroidal oestrogens.

estradiol like estradiol- cypionate (estradiol cyclopentylpropionate), -valerate, -benzoate and -propionate are effective orally and are used preferentially. In veterinary medicine, estradiol cypionate is more commonly used than other semi-synthetic preparations.

Pharmacological effects

The mechanism of action and biological effects of exogenous estradiol and its semi-synthetic preparations are essentially similar to those of endogenous estradiol. Semi-synthetic estradiol releases the parent molecule on hydrolysis in body, which in turn is responsible for the pharmacological effects. Etherification only delays the absorption and prolongs the biological half-life.

Pharmacokinetics

The detailed pharmacokinetic data on estradiol and various esters of estradiol in domestic animals are lacking. Natural estradiol is inactive orally in spite of good absorption due to rapid metabolism in the liver. It can be administered parenterally (SC) to achieve a brief duration of action (t½ 15-30 minutes). Estradiol in vegetable oils such as peanut or corn oil delays absorption from injection site and has relatively longer action (1-3 days). In contrast to the estradiol, the esters of estradiol are effective both orally and parenterally. Esterified estradiols are slowly absorbed from the IM injection site and exert prolonged action. Estradiol benzoate has shorter duration but estradiol cypionate has longer duration of action. A single therapeutic dose of estradiol cypionate may be absorbed over several weeks following IM injection.

After administration, estradiol is released from esters and transported mainly by sex-steroid binding globulin and also albumin to different parts of body. About 2-3% of estradiol remains free. Estradiol is metabolised mainly in liver and metabolises are excreted in urine (mainly) and bile. Considerable enterohepatic circulation occurs due to deconjugation in intestine and reabsorption.

Side effects/Adverse effects

The adverse effects of estradiol have been documented mainly in small animals. In dogs and cats, all preparations of estradiol may cause blood dyscrasias and bone marrow depression. Estradiol, particularly if used repeatedly, may cause aplastic anaemia and increase risk of cystic endometrial hyperplasia and pyometra in bitches. In cattle, prolonged oestrus, genital irritation, precocious development and follicular cysts, and decreased milk yield may develop after estradiol therapy. Other adverse effects in different species may include vaginal and rectal prolapse, abortion, bone fracture and inhibition of pituitary functions.

Contraindications and precautions

Estradiol is contraindicated in pregnant animals as it can induce abortion. High oestrogen levels may cause foetal genitourinary malformations and induce bone marrow depression. Some practitioners advise not to use estradiol preparations in cats. Estradiol therapy should not be used for long periods on dogs because of bone marrow depression. Estradiol should not be used is oestrogen-dependent neoplasm. Oestrogens are highly efficacious for most of their therapeutic purposes, but decision regarding their use should always depend on the risk-to-benefit ratio for each patient.

Drug interactions

Estradiol may alter protein binding and/or biotransformation of glucocorticoids and potentiates their biological activity. Oral anticoagulant activity may be decreased if estradiol is administered concurrently. Several microsomal enzyme inducers are reported to decrease activity of estradiol by enhancing its biotransformation.

Clinical uses

In veterinary medicine, estradiol and its semi-synthetic preparations are used mainly to nullify misalliance in bitches and to treat anoestrus in cattle and mares. A single dose of estradiol shortly after misalliance in bitches is effective because the exogenous estradiol interferes with tubular transport of ova in the oviduct leading to zygotic-degeneration in addition to causing hypertrophy of uterine mucosa. In cattle, oestrogens are used to treat chronic endometritis and appear to be effective at low dosages. The oestrogens must not be used in acute uterine infections because they enhance the absorption of bacterial toxin. Occasionally, oestrogens are used in bitches for treatment of problems associated with spaying (e.g. urinary inconsistence, vaginitis and dermatitis). In males, oestrogens are used in the treatment of excess libido, anal adenoma and prostrate hyperplasia. For anal adenomata, long acting estradiols are preferred.

Estradiol is combined with progesterone or testosterone for parenteral (implant) administration to promote growth in calves, heifers or steers (Chapter 75). The combination of estradiol with progesterone is also used by some veterinarians to induce lactation in infertile dairy cows or heifers, but this practice in not allowed by several countries. Other possible uses of estradiol include treatment of persistent corpus luteum in cows, stimulation of uterus for expulsion of retained placenta and mummified foetus, and as an abortifacient agent in cattle and bitches. Estradiol is marketed in a number of dosage forms including oral, transdermal, topical, injectable and vaginal preparations for use in humans. Estradiol cypionate, used largely in veterinary practice, is available in injectable form.

Dose

Estradiol cypionate

For pregnancy avoidance after mismating

Dogs : 0.02 – 0.04 mg/kg, IM, once during oestrus or within 72 hours of mismating.

Cats : 0.125 – 0.25 mg (total), IM, between 40 hours and 5 days after mating.

For treatment of anoestrus or induction of oestrus

Cattle : 3 – 5 mg (total), IM.

Sheep and pigs: 0.5 – 1 mg, IM.

Horses : 10 mg (total), IM. It may result in oestrus 2-3 days after treatment, if given during the non-breading season.

For endometritis/retained placenta/mummified foetus

Cattle : 10 mg (total), IM.

For persistent corpus luteum

Cattle : 4 mg (total), IM.

Other drugs

Other naturally occurring steroidal oestrogenic drugs include estrone, equilin and equilenin. As discussed earlier, estrone is one of the natural oestrogens secreted by the ovary. Equilin and

equilenin are the two oestrogens produced by the pregnant mare which are unique to Equidae. Both equilin and equilenin have unsaturated B rings and are obtained from pregnant mare's urine. A commercial preparation named 'Premarin' containing estrone (>50%), equilin (15-25%) and equilenin is available for use in women.

3. Synthetic Steroidal Oestrogens

Ethinylestradiol

Ethinylestradiol (Ethynylestradiol, EE) is an orally effective synthetic oestrogen preparation. It is prepared by substitution at C17 of the estrane steroid with an ethinyl group. After administration, ethinylestradiol is cleared much more slowly than is estradiol due to its decreased hepatic metabolism. Unlike estradiol, ethinylestradiol is bound extensively to serum albumin but not to sex-steroid binding globulins. Like other steroidal oestrogen preparations, adverse effects such as inhibition of pituitary function, anaemia and thrombocytopenia, and cystic endometrial hyperplasia in bitches and squamous metaplasia of prostrate in male dogs have been documented with high doses. In human medicine, the most common use of ethinylestradiol is in the oestrogen-progestogen combined oral contraceptive pills.

Dose

For prostratic hyperplasia and anal adenoma

Dogs : 0.05 – 0.1 mg (total), PO, daily. If feminisation occurs, the therapy may be stopped and restarted with half original dose.

Mestranol

Mestranol is the 3-methyl ether of ethinylestradiol. It is a biologically inactive prodrug that acts by getting converted to ethinylestradiol in the liver. The oestrogenic effects are produced by ethinylestradiol. Mestranol is occasionally used in human medicine as oestrogenic compound in some oral contraceptives.

Quinestrol

Quinestrol (Ethinyl estradiol 3-cyclopentyl ether) is a synthetic oestrogen. It is used in human patients mainly in hormone replacement therapy, and occasionally in treatment of breast cancer and prostate cancer.

II. Non-Steroidal Oestrogens / Synthetic Non-Steroidal Oestrogens

Diethylstilbestrol

Diethylstilbestrol (Diethylstilboestrol, stilboestrol, DES) is a synthetic non-steroidal oestrogen. It is derived from the basic stilbene nucleus and resembles estradiol when viewed in the trans conformation. Diethylstilbestrol was first synthesized in 1938 and introduced in 1940s as oestrogen-replacement therapy for various oestrogen deficiency states. The DES is still widely used in veterinary medicine, but its use has declined in human medicine mainly due to availability of better drugs.

Pharmacological effects

Diethylstilbestrol possesses pharmacological effects similar to those of estradiol. In addition, it possesses antineoplastic activity against some types of neoplasias (e.g. perianal gland adenoma and prostatic hyperplasia).

Pharmacokinetics

Diethylstilbestrol is well absorbed after oral administration, but unlike estradiol it is slowly metabolised by the liver. This leads to longer duration of action. Diethylstilbestrol is mainly excreted in a glucuronide form in the urine and faeces.

Side effects/Adverse effects

Side/Adverse effects of DES are similar to those of estradiol. Blood dyscrasias are more common in old animals and if high dosages are used. Chronic DES toxicity may be characterised by a normochromic-normocytic anaemia, thrombocytopenia and neutropenia. Dermatological disorders may also be observed in dogs. In humans, DES has been linked to an uncommon cancer of the vagina or cervix (called clear cell adenocarcinoma) in a small number of daughters of women who used DES during pregnancy. Such daughters are commonly referred to as "DES daughters".

Contraindications and precautions

Diethylstilbestrol is contraindicated in pregnancy because it may cause foetal malformations of the genitourinary system. The DES has been found to be carcinogenic at low levels in some laboratory animals hence its use is prohibited in food animals. Overdosage may cause severe inhibition of pituitary function, anaemia and thrombocytopenia.

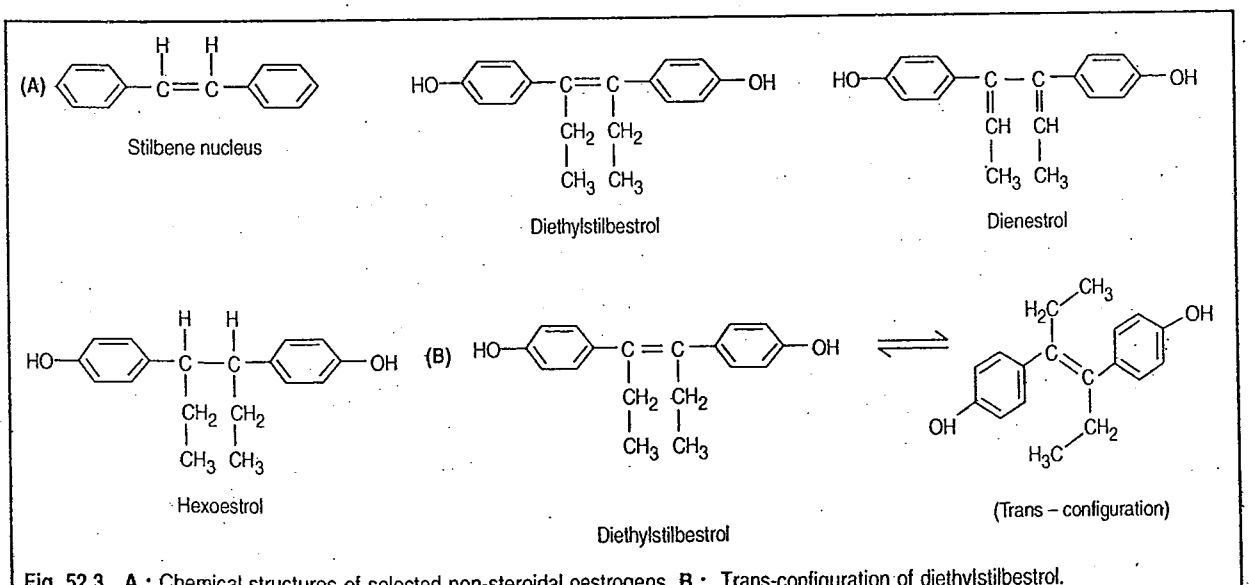


Fig. 52.3. **A :** Chemical structures of selected non-steroidal oestrogens. **B :** Trans-configuration of diethylstilbestrol.

Drug interactions

Drug interactions of DES are similar to those of estradiol preparations.

Clinical uses

Diethylstilbestrol is used mainly in the treatment of misalliance in bitches and in oestrogen responsive incontinence in spayed female dogs. However, its efficacy against prevention of mismating is doubtful, especially when it is used alone. Diethylstilbestrol is used occasionally in veterinary medicine for the treatment of certain oestrogen-responsive neoplasms like perianal gland adenoma and prostatic hyperplasia. Diethylstilbestrol has been shown to be effective in the control of aggression in bitches after spaying although potential adverse effects may limit its use.

Dose

For pregnancy avoidance after mismating

Dogs : 0.1 – 1 mg (total), PO, once daily for 5 days, if animal is presented 1-2 days after coitus.

: 1 –2 mg (total), PO, once daily for 5 days after estradiol cypionate therapy, if animal is presented 5 days after coitus.

For treatment of prostatic hyperplasia and anal adenoma

Dogs : 0.1 – 1 mg (total), PO, once daily or every alternate day.

For treatment of oestrogen responsive incontinence

Dogs : 0.1 – 1 mg (total), PO, once daily for 3-5 days, then 1 mg once weekly.

Other synthetic non-steroidal oestrogens

Some other synthetic non-steroidal oestrogens occasionally used in human and/or veterinary medicine include hexoestrol, dienestrol, benzestrol, methallenoestrol, chlorotrianisene, fosfestrol and zeranol. Some of these (e.g. dienestrol) are used mainly as vaginal cream. Chlorotrianisene is converted into a more active form in the body and is stored in fat depots from where it is slowly released. Fosfestrol is a synthetic oestrogen used as an antineoplastic agent. Zeranol (α-zearalanol), a non-steroidal oestrogen obtained from a mycotoxin, is used occasionally to promote growth in cattle and sheep.

II. DRUGS USED IN EXCESS OF OESTROGENS / ANTI-OESTROGENIC DRUGS

A. Oestrogen Receptor Inhibitors/Anti-oestrogens

Oestrogen receptor inhibitors are compounds which compete with oestrogens for binding sites on oestrogen receptors in the target sites and do not allow all biological activity of oestrogens. These agents are used therapeutically for their anti-oestrogenic activity, but many of these can produce antagonistic, agonistic, or partial agonistic actions depending on species, target organ, type of response and hormonal status of the animal. These compounds are used mainly in human medicine and have limited use in animals. However, they can be used to reduce the size of oestrogen-dependent tumours in some animals.

1. Mixed Oestrogen Receptor Antagonist-Agonists

Mixed oestrogen receptor antagonist/agonist, commonly called selective estrogen receptor modulators (SERMs), are a group of pharmacologically related drugs with antagonistic as well as partial agonistic activity at oestrogen receptors. These drugs possess anti-oestrogenic activity in some organs such as mammary tissue and oestrogenic activity in some other tissues such as reproductive tract and bones. These are largely used in treatment of breast/mammary tumour.

Clomifene

Clomifene (Clomiphene, Clomid, Clomifert) citrate is a non-steroidal compound that is structurally related to diethylstilbestrol. It functions as selective oestrogen receptor modulator with antagonistic as well as partial agonistic activity at oestrogen receptors. Trans-isomers usually possess antioestrogenic activity, while cis-isomers display oestrogenic activity. Clomifene is a racemic mixture of both trans (enclomifene) and cis (zuclomifene) isomers.

Pharmacological effects

Clomifene blocks hypothalamic and anterior pituitary oestrogen receptors and reduces feedback inhibitory effect of oestrogens on the secretion and release of gonadotropins. This increases production and secretion of gonadotropins from anterior pituitary. Increase in gonadotropin activity induces ovulation. This anti-oestrogenic action of clomifene is very prominent in women in which it induces ovulation, causes enlargement of ovaries, and reduces or regresses oestrogen-induced vaginal epithelial keratinisation. Large dose may lead to ovarian cysts. In rats, however, clomifene produces weak oestrogenic action, decreases pituitary gonadotropins secretions and acts as contraceptive. Status of clomifene in veterinary species is not clear.

Clinical uses

Clomifene is used primarily in human medicine for the treatment of female infertility due to lack of ovulation (anovulatory sterility). It is also indicated for use in low sperm count in males with variable results. In veterinary medicine, it is occasionally used in dogs. Common adverse effects associated with the use of clomifene in human patients include vasomotor flushes, abdominal discomfort, visual blurring (dose-dependent), and/or reversible ovarian enlargement and cyst formation.

Dose

Dogs : 25 mg/kg, PO, once daily.

Tamoxifen

Tamoxifen is an antioestrogen that is structurally related to diethylstilbestrol and clomifene. It is used mainly in the treatment of oestrogen-dependent breast cancer in women. In veterinary medicine, tamoxifen is used occasionally as an adjunct in the treatment of mammary gland carcinoma in bitches.

Mechanism of action

Tamoxifen is a mixed antagonist/agonist, but the type of effect produced varies with the species and tissue. As an antagonist, it competitively binds to oestrogen receptors on mammary tumours and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits oestrogen effects. In mammary/breast tumours, tamoxifen causes cells to remain in the G_0 and G_1 phases of the cell cycle. Because it prevents cancerous cells from dividing but does not cause cell death, tamoxifen produces a cytostatic rather than cytocidal effect. Tamoxifen is metabolized into compounds which also bind to and block the oestrogen receptor but do not activate it. Tamoxifen also may increase release of gonadotropin-releasing hormone (GnRH).

Pharmacological effects

Tamoxifen itself is a prodrug, having relatively little affinity for its target oestrogen receptor. It is metabolized in the liver by the cytochrome P450 isoform CYP2D6 and CYP3A4 into active metabolites such as 4-hydroxytamoxifen and N-desmethyl-4-hydroxytamoxifen (endoxifen), which have 30-100 times more affinity for the oestrogen receptor than tamoxifen itself. Similar to clomifene, tamoxifen possesses both oestrogen and antioestrogen actions. Tamoxifen appears to have, at least in parts, a direct oestrogenic effect on the reproductive tract of bitches, but possesses antioestrogenic effect on the mammary tissues. The effects of tamoxifen on proliferation of mammary tumour cells appear to result largely from competitive oestrogen receptors blockade. The oestrogenic effects of tamoxifen to the reproductive tract may produce vulvar oedema and sanguineous vaginal discharge in bitches. In bone, the oestrogenic properties of tamoxifen inhibit osteoclasts and prevent osteoporosis, a beneficial side effect.

Side/Adverse effects

Adverse effects have not been clearly documented in animals. In human patients, tamoxifen has been reported to cause increased tumour pain. The partial agonist action of tamoxifen on the endometrium has been linked to endometrial cancer in some women. Tamoxifen treated breast cancer patients show evidence of reduced cognition and libido.

Contraindications and drug interactions

Use of tamoxifen is contraindicated in pregnant animals. Tamoxifen is a potent cytochrome P-450 enzyme inhibitor; so it may show interaction with drugs being metabolised by this enzyme system.

Clinical uses

Tamoxifen is widely used in the treatment of oestrogen-dependent breast cancer in women. It has also been used in women to induce ovulation by stimulating release of Gn-RH from the hypothalamus. In veterinary practice, tamoxifen is used occasionally in bitches as an adjunct in the treatment of mammary gland adenocarcinoma.

Dose

Dogs : 1 -2 mg/kg, PO, 2 times daily.

Toremifene

Toremifene (Chlorotamoxifen) is structurally and pharmacologically related to tamoxifen, but differs from tamoxifen by chlorination of the ethyl side chain. Its antioestrogenic potency is less than tamoxifen. Toremifene citrate has been approved for use in advanced (metastatic) breast cancer. It is also being evaluated for prevention of prostate cancer.

Other drugs

A large number of mixed oestrogenic receptor antagonist/ agonists are either available commercially or are undergoing clinical trials for use in human medicine. These drugs include cyclofenil, afimoxifene, arzoxifene, bazedoxifene, lasofoxifene, nafoxidine, ormeloxifene and raloxifene. Cyclofenil has action similar to that of clomifene, but is less used nowadays. Ormeloxifene (also known as centchroman) has clinical use as a contraceptive, but is used also in treatment of dysfunctional uterine bleeding. Raloxifene is used orally in the prevention of osteoporosis in postmenopausal women. Afimoxifene, arzoxifene, bazedoxifene, lasofoxifene and nafoxidine are in advance phases of clinical trials.

2. Selective Oestrogen Receptor Antagonists

Fulvestrant

Fulvestrant is an oestrogen receptor antagonist with no agonist effects. In addition to oestrogen receptor blockade, fulvestrant produces anti-oestrogenic effects by down-regulating and degrading the oestrogen receptor. Accordingly it also sometimes included in a special class of anti-oestrogenic drugs called "selective oestrogen/estrogen receptor down-regulator (SERD). Fulvestrant has been approved for use in human medicine for the treatment of hormone receptor positive metastatic breast cancer in postmenopausal women with disease progression following anti-oestrogen therapy. Gastrointestinal symptoms (including nausea, vomiting, constipation, diarrhoea and abdominal pain), headache, back pain, vasodilatation (hot flashes) and pharyngitis are important side/adverse effects observed in women. Fulvestrant is administered as a once-monthly injection.

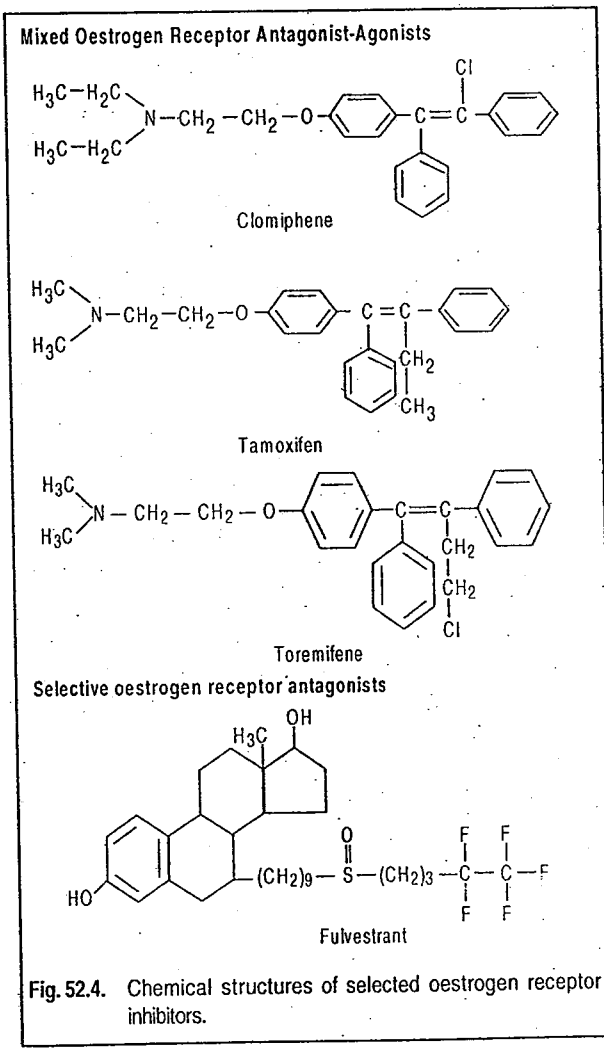


Fig. 52.4. Chemical structures of selected oestrogen receptor inhibitors.

B. Aromatase Inhibitors/ Oestrogen Synthesis Inhibitors

Several groups of drug can be used to inhibit the biosynthesis of endogenous oestrogens. These include continual administration of gonadotropin-releasing hormone (GnRH) or use of long-acting GnRH agonists (see Chapter 49). These agents can block ovarian synthesis of oestrogen, but do not prevent peripheral oestrogen synthesis. A recent group of anti-oestrogenic drugs called aromatase inhibitors block oestrogen biosynthesis from all precursors.

1. Selective Aromatase Inhibitors

Anastrozole

Anastrozole belongs to the group of drugs which block the enzyme aromatase, which is responsible for converting androgens to oestrogens in peripheral tissues. Anastrozole prevents the steroidal aromatase from producing oestrogens by competitive, reversible binding to the heme of its cytochrome P-450 unit. Inhibition of this essential enzyme in the biosynthesis of oestrogens leads to profound decline in the levels of oestrogen (hypoestrogenism). The action of anastrozole is specific, as it does not reduce production of corticosteroids.

Anastrozole has been recommended in the treatment breast cancer, especially in those women in which lesions are found to be oestrogen receptor positive. The drug is also used in men to reduce levels of estradiol, which is associated with benign prostatic hyperplasia and gynaecomastia. Occasionally, it is used also in children in the management of growth disorder to stop or slow the onset of puberty and in the treatment of moderate-to-severe pubertal gynaecomastia. Anastrozole is well tolerated, but incidence of weakening of bones and osteoporosis has been reported after long term use. Due to this reason, anastrozole is often prescribed along with drugs such as bisphosphonates which regulate osteoporosis. Other side effects may include signs and symptoms of hypoestrogenism.

Letrozole

Letrozole is another selective aromatase enzyme inhibitor used for the treatment of hormonally-responsive breast cancer after surgery. Similar to anastrozole, it binds reversibly to the aromatase enzyme through competitive inhibition. The anti-oestrogen action of letrozole has been shown to be useful in pre-treatment for termination of pregnancy, in combination with misoprostol. It has also found some usage in the treatment of endometriosis. The most common side effects are sweating, hot flashes, arthralgia and fatigue.

Exemestane

Exemestane is another selective aromatase enzyme inhibitor with pharmacological effects similar to those of anastrozole and letrozole. However, unlike anastrozole and letrozole, exemestane is structurally related to androstenedione and its inhibitory action on aromatase enzyme is irreversible. Due to the structural resemblance to natural substrate androstenedione, it acts as a false substrate for the aromatase enzyme and is processed to an intermediate that binds irreversibly to the active site of the enzyme causing its permanent inactivation, an effect known as "suicidal inhibition". The oestrogen suppression rate for exemestane varies from 85% for estradiol to 95% for estrone. It is mainly used in human medicine in the adjuvant treatment of oestrogen responsive breast cancer in postmenopausal women. Exemestane is also indicated for the treatment of advanced breast cancer in postmenopausal women whose disease has progressed following tamoxifen therapy. Side effects may include signs and symptoms of hypoestrogenism.

Other drugs

Fadrozole, formestane and vorozole are other selective aromatase enzyme inhibitors used in human medicine. Their pharmacological properties and clinical usage are similar to those of other aromatase inhibitors discussed above.

2. Non-Selective Aromatase Inhibitors

Aminoglutethimide

Aminoglutethimide is a non-selective anti-steroid drug that blocks production of many steroidal hormones derived from cholesterol. It has two important types of action: blockade of aromatase enzyme and inhibition of cholesterol side-chain cleavage enzyme P450scc. As discussed above, blockade of aromatase enzyme inhibits production of oestrogens from androstenedione and testosterone. Inhibition of P450scc blocks the conversion of cholesterol to pregnenolone and consequently decreases synthesis of all hormonally active steroids. At low doses, aminoglutethimide is only an effective inhibitor of aromatase, but at higher doses, it effectively blocks P450scc as well.

Aminoglutethimide has oral bioavailability of more than 95%. It is metabolized in liver and excreted largely in urine. Half-life in human beings is reported to be about 12 hours. Aminoglutethimide has been used in conjunction with other drugs to decrease hypersecretion of hydrocortisone in human patients with Cushings's syndrome (see also Chapter 51). Because of its action to inhibit aromatase, aminoglutethimide has also been recommended for the treatment of hormone sensitive (oestrogen and progesterone) metastatic breast cancer in women. Important side/adverse effects related to aminoglutethimide use include

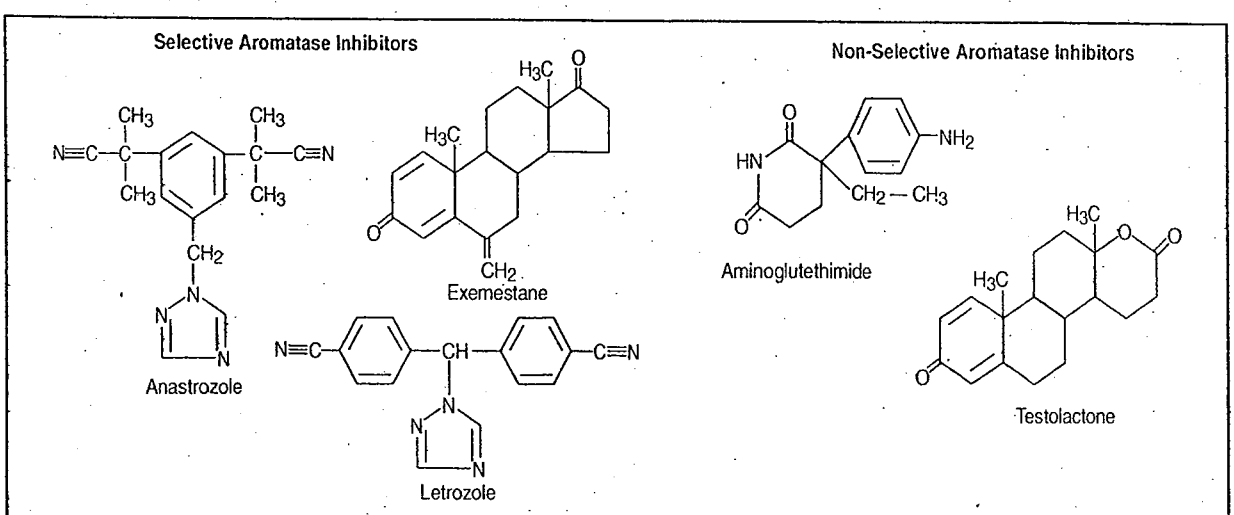


Fig. 52.5. Chemical structures of important aromatase inhibitor anti-oestrogen drugs.

skin rashes, hepatotoxicity and signs and symptoms related to hypoadrenocorticism and hypoestrogenism. It may also cause hypothyroidism. Aminoglutethimide has limited therapeutic application in veterinary medicine.

Testolactone

Testolactone is a synthetic steroidal aromatase enzyme inhibitor derived from progesterone. It inhibits aromatase enzyme by a non-competitive and irreversible interaction. It is structurally distinct from gonadal hormones in possessing a six-member lactone ring in place of the usual five-member carbocyclic D-ring in the steroid nucleus. Despite some similarity to testosterone, the principal action of testolactone is reduction in oestrogen biosynthesis, with no androgenic and other hormonal effects. It is primarily used as anti-oestrogenic drug in treatment of various types of breast cancer in women.

PROGESTOGENS

The progestogens (progestins, progestagens, gestagens, progesterones) are a group of naturally occurring steroidal hormones. These include progesterone, the primary progestational hormone, and 17α-hydroxyprogesterone, a minor hormone. For practical purposes, progesterone is considered as the single important natural progestogen. The progesterone is secreted by the corpus luteum of cycling animal and by corpus luteum and placenta of some species during pregnancy. The testes in males and adrenal cortex in both sexes also secrete small amounts of progesterone. Progesterone is needed for the maintenance of pregnancy in all species whether supplied by the corpus luteum or placenta, or both.

Synthesis, Release, Transport and Fate

Synthesis and release: The progesterone is characterized by a basic 21-carbon skeleton, called a pregnane skeleton (C21). It is synthesised mainly from cholesterol, but to a lesser extent also from acetyl coenzyme A. It is produced by the corpus luteum that has capability to uptake and store cholesterol under the influence of LH. During the luteal phase of the cycle, large amounts of progesterone is formed and secreted by corpus luteum. Its production declines a few days before the next cycle. During pregnancy, the placenta secrets lots of progesterone that usually continues till term.

Transport and fate: Progesterone circulates in blood mainly carried on red blood cell membrane and by plasma albumin and specific progesterone binding globulins. Within few minutes after secretion, almost all progesterone is degraded mainly in the liver to other steroids which have no progesteronic effect. The major end product of progesterone metabolism is pregnanediol, which is excreted as a glucuronide in urine. Progesterone is the precursor of the mineralocorticoid aldosterone, glucocorticoid hydrocortisone and cortisone, and after conversion to 17-hydroxyprogesterone (another natural progestogen) to androstenedione. Androstenedione can be converted to testosterone, estrone and estradiol (Fig. 48.2). The physiological half-life of progesterone is only 20 to 36 minutes in cows.

Mechanism of Action

Progesterone is a highly lipid-soluble compound. Like oestrogens, progesterone enters the target cell and combines with specific receptor protein called the progesterone receptor (PR). The progesterone-receptor complex is then transported into the nucleus, where it interacts with nuclear chromatin to induce synthesis of specific proteins via messenger RNA synthesis. In addition, progesterone is a highly potent antagonist of the mineralocorticoid receptor (MR), the receptor for aldosterone and other mineralocorticosteroids.

The progesterone receptor is expressed in the female genital tract, mammary gland, pituitary and some regions of brain. In general, progesterone receptor has a limited tissue distribution than do oestrogen or other steroid hormone receptors. In many cells, oestrogens have been shown to induce progesterone receptor, whereas progesterone represses oestrogen receptors and induces local degradation of estradiol.

Physiological Effects

The main physiological role of progesterone is preparation of uterus for nidation and maintenance of pregnancy. Though reported to as a sex hormone, progesterone affects many tissues/ systems in the body including reproductive tract, endocrine system, brain cells, fat metabolism, water balance, energy production and thermogenesis, and immune system The effects of progesterone on reproductive tract and some other organs is usually seen only after oestrogen has primed the target tissue.

Reproductive tract: Progesterone acts on uterus to cause quietening of the myometrium by decreasing the frequency and intensity of uterine contraction. Progesterone promotes secretory changes in uterine endometrium and mucosal lining of fallopian tubes in second phase of the ovarian cycle. This secretion prepares the uterus for implantation of the fertilised ovum and provides nutrition to it. Progesterone production by the corpus luteum and/or placenta may be essential for pregnancy maintenance due to its ability to inhibit cell-mediated responses involved in tissue rejection. During implantation and gestation, progesterone appears to decrease the maternal immune response to allow for the acceptance of the pregnancy. The immunosuppressant activity of progesterone is very similar to that of glucocorticoids. In cervix, progesterone converts thin cervical secretions induced by oestrogens to thick and tenacious secretions which occlude sperm penetration. Overall, progesterone favours pregnancy; abortion generally ensues if progesterone is lacking or deficient during pregnancy.

Mammary gland: Progesterone causes development and proliferation of lobule-alveolar system in the mammary gland during pregnancy. However, progesterone does not cause the alveoli actually to secrete milk because milk is secreted only after the prepared mammary gland is further stimulated by prolactin from the anterior pituitary gland.

Metabolism: Progesterone favours an economy of body metabolism and has anabolic effects because appetite is stimulated during pregnancy and feed is utilised more efficiently. Progesterone stimulates lipoprotein lipase activity and it seems to enhance fat deposition. Although progesterone can weakly promote sodium and water retention due to its direct effect on renal tubules, it actually diminishes the effect of more potent aldosterone, thereby causing net loss of sodium and water from the body. Progesterone in large quantities can enhance sodium, chloride and water reabsorption from distal tubules of kidneys. Progesterone also exerts an anti-glucocorticoid effect by acting as a ligand for glucocorticoid receptors.

Pituitary and CNS: Progesterone in conjunction with basal secretion of estradiol acts as a negative feedback hormone during the ovarian cycle suppressing LH and FSH secretion, oestrus and ovulation. Therefore, as long as the corpus luteum is present, final growth and maturation of follicles are prevented. Recent studies have indicated that progesterone promotes myelin sheath repair in the brain.

Other effects: High circulating levels of progesterone (during pregnancy) appears to have a sedative effect. Progesterone also enhances the body temperature (0.5 °C) seen during luteal phase by resetting hypothalamic thermostat and increasing heat production. In some species, progesterone primes behavioural centres in the brain and so allows estradiol to induce behavioural oestrus. Progesterone also appears to facilitate the behavioural receptivity in females. It acts as an antiinflammatory agent and regulates the immune response. It reduces spasm and relaxes smooth muscle.

DISORDERS OF PROGESTERONE

Deficiency of Progesterone

Deficiency of natural progesterone is not a common problem in animals, but has become more frequent in human beings. Progesterone deficiency if occurs, may result in oestrogen dominance, a condition where individual may have deficient, normal or excessive oestrogen but has little or no progesterone to balance its effects in the body. Follicular cysts in animals may produce progesterone deficiency with signs of hyperoestro-genism, primarily expressed as intense oestrus behaviour. Anovulatory cycles in women cause low progesterone levels in the pre-menopausal years. Excessive exposure to xenoestrogens through pesticides, plastics, industrial wastes, household materials etc. are enhancing incidence of oestrogen dominance in both human beings and animals. Stressful living in human beings also leads to oestrogen dominance because hydrocortisone and progesterone may compete for common receptors in cells and excessive hydrocortisone release during stress impairs progesterone activity.

Progesterone deficiency (sometimes also called hypoluteoidism) has been incriminated as a cause of abortion in mares, queens, bitches and some other species. Repeated abortions in the later-half of pregnancy in the absence of infections may suggest a progesterone deficiency. As oestrogens and progesterone has balancing effects on the uterus; oestrogen dominance in absence of progesterone causes uterus to grow in size. Oestrogen dominance in women generally causes discomfort, heavy menstrual bleeding and development of fibroids. It has also been associated with water retention, headache, breast swelling, fatigue, bloating, mood swings, leg cramps, anaemia, endometriosis, loss of libido and poor sleep pattern. Oestrogen dominance in presence of progesterone deficiency has been reported to increase risk of breast cancer in women because the down regulating action of progesterone on some genes (e.g. BCL-2) responsible for oestrogen stimulated rapid growth of breast cells is lost.

Excess of Progesterone

Literature on excess of progesterone and related abnormalities in animals is scarce. High plasma progesterone levels are observed in luteal cysts in animals. Excessive progesterone may prevent final growth and maturation of follicles. Too much progesterone in women has been associated with tiredness and sedation. Excess progesterone down-regulates oestrogen receptors.

TREATMENT OF PROGESTOGEN DISORDERS

Disorders of progestogen can be treated by using a wide variety of drugs possessing either progesterone-like activity or anti-progesterone activity. These drugs are commonly used in human medicine, but have limited clinical use in animals.

Classification

Drugs used to treat progestogen disorders have been broadly classified into two classes: drugs used in deficiency of progestogens and drugs used in excess of progestogens.

I. Drugs used in deficiency of progestogens

Depending on the chemical structure, drugs used in deficiency of progestogens have been divided into following groups:

1. Natural progestogens
   e.g. progesterone.
2. Progesterone derivatives
   e.g. medroxyprogesterone acetate, megestrol, melengestrol acetate, hydroxyprogesterone, proligestone, flugestone, chlormadinone, dydrogesterone and medrogestone.
3. 19-Norprogesterone derivatives
   e.g. nomegestrol, trimegestone, norgestomet, gestonorone and promegestone.
4. Testosterone or 19-Nortestosterone derivatives

a. Older progestogens
   e.g. ethisterone, norethisterone, norethynodrel, ethynodiol diacetate, lynestrenol, allylestrenol, norgestrel, levonorgestrel and norgestrienone.

b. Newer progestogens
   e.g. desogestrel, norgestimate, gestodene, etonogestrel and dienogest.

5. Miscellaneous progestogens
   e.g. altrenogest, cyproterone, tibolone, drospirenone, quingestanol and norelgestromin.

II. Drugs used in excess of progestogens

1. Progesterone receptor inhibitors

a. Selective progesterone receptor antagonists
   e.g. mifepristone.

b. Mixed progesterone receptor antagonist/agonists
   e.g. ulipristal and asoprisnil.

2. Progesterone synthesis inhibitors
   e.g. epostane.

I. DRUGS USED IN DEFICIENCY OF PROGESTOGENS

The deficiency of naturally occurring progestogens can be treated by using a large number of drugs obtained from natural, semi-synthetic or synthetic sources. Physiologically, the term progestogen or progestin refers to the naturally occurring hormone progesterone, but in pharmacology the term includes all natural, semi-synthetic or synthetic compounds which have progestational activity. All types of progestogen, irrespective of their source, act on progesterone receptors and can convert the oestrogen-primed endometrium to secretory and maintain pregnancy in animal after conception.

1. Natural Progestogens

Progesterone

Progesterone is the major natural progestogen secreted by the ovarian corpus luteum and placenta in females. Progesterone is available commercially but it less used in therapeutics due to its short half-life and inactivity after oral administration. Progesterone is rapidly metabolised by liver enzymes following gastrointestinal absorption. Progesterone may be used in oily solution that delays the absorption of the steroid from injection site and gives longer duration of action.

Progesterone supplementation is used to support pregnancies regarded as at risk (to prevent abortion and embryonic deaths). Progesterone is also administered to cattle by using a progesterone-releasing intravaginal device for synchronisation of oestrus and induction of ovulation in anoestrus conditions. It is also recommended for use in bitches to suppress oestrus but for short duration only. Progesterone is occasionally combined with estradiol in the formulation of tablet implant to enhance live-weight gain in steers (See Chapter 75). The adverse effects and contraindications of progesterone are similar to those mentioned for medroxyprogesterone.

Dose

Dogs : 1 – 3 mg/kg, SC or IM, for progesterone treatment of short duration.

Cats : 0.2 – 2 mg/kg, SC or IM, for progesterone treatment of short duration.

For synchronisation of oestrus/induction of ovulation in anoestrus

Cattle : 1 device, by intravaginal administration. The device is removed after 7-12 days. Prostaglandin $F_{2\alpha}$ or analogue may be administered before removal of device to improve the accuracy of synchronisation.

2. Progesterone Derivatives

The synthetic derivatives of progestogens contain 21-carbon skeleton of progesterone and, therefore, are highly selective with almost pure progestational activity. These compounds have a spectrum of activity very similar to that of endogenous hormone progesterone, but generally have a longer half-life and possess physiological activity in much smaller quantities. Some of these are orally active, which increases their potential use in veterinary practice. These agents are used mainly to control or suppress oestrus and ovulation.

Medroxyprogesterone acetate

Medroxyprogesterone acetate (Medroxyprogesterone 17-acetate, acetomethylprogesterone, methylacetoxy progesterone) is a synthetic progestogen, which is structurally related to the progesterone. Compared to medroxyprogesterone acetate (MPA), the parent compound medroxyprogesterone (MP) is approximately 100 fold less potent as a progestogen and is not used as a drug. However, in routine practice the two names i.e. medroxyprogesterone acetate and medroxyprogesterone are used as synonyms and serve the same purpose. Medroxyprogesterone acetate occurs as an odourless, white to off-white crystalline powder that is insoluble in water. It is used widely in both human and veterinary medicines for various gynaecological problems.

Pharmacological effects

The mechanism of action and pharmacological effects of MPA are generally the same as the physiological actions described for progesterone. It transforms proliferative endometrium to secretory endometrium, enhances myometrium hypertrophy and inhibits spontaneous uterine contractions. It produces dose-dependent inhibitory effect on the secretion of pituitary gonadotropins. In addition, MPA is anti-oestrogen and can also decrease plasma testosterone level in males. In males, MPA represses spermatogenesis and increases the basal levels of growth hormone. Medroxyprogesterone acetate also possesses adrenocorticoid effect in animals and can suppress ACTH and hydrocortisone release. It is reported that MPA has antineoplastic activity against endometrial carcinoma and renal carcinoma in human beings but its efficacy is doubtful.

Pharmacokinetics

The pharmacokinetics data on MPA in domestic animals are limited. In human beings, MPA is well-absorbed orally and through intramuscular injection. Intramuscular MPA is released slowly peaking at 2–4 hours with very slow decline over weeks. It binds to albumin in the blood and is metabolized primarily through the liver via hydroxylation and conjugation. It is more resistant to hepatic metabolism than the progesterone due to substitution at the 6 position of ring B. The duration of action of MPA is about 30 days when used to treat behaviour disorders in cats. The metabolism is thought to be primarily hepatic and elimination is generally via urine as conjugates. The half life of MPA in humans is about 24 hours for an oral dose and 50 days for an intramuscular injection.

Side effects/Adverse effects

Progestogen therapy can cause serious adverse effects in animals. Important adverse effects related to MPA and other progestogens in dogs and cats include increased appetite and/or thirst, depression, lethargy and temperament changes. Like other progestogens, MPA stimulates the proliferative and secretory activity of uterine endometrium leading to cystic endometrial hyperplasia, mucometra or pyometra. Subcutaneous injection of MPA and other progestogens may cause hair discolouration, localised alopecia and thinning of skin. If administered by SC route, it is better to use the inguinal area to avoid these manifestations. Adrenocortical depression, enlargement of mammary glands and tumours may be seen in some bitches. In males, there may be temporary inhibition of spermatogenesis. Blood sugar may rise and diabetes may be precipitated by long-term use of MPA and other progestogens due to anti-insulin effect of these drugs. In dogs, acromegaly and increased growth hormone levels have also been reported.

Contraindications and precautions

Progestogens should not be used in animals with a history of vaginal discharge or pyometra as it may get precipitated with steroidal therapy. The MPA may antagonise the hypoglycaemic effects of insulin and therefore should not be given to diabetic animals. Preparations containing progestogens should not be used in sexually immature animals or animals intended for breeding. Women of childbearing age should not handle progestogens preparations. If required, operators should wear impervious gloves and protective clothing. As all progestogen preparations possess serious adverse effects, the potential risks versus benefit should be weighed before the therapy is instituted. Because MPA can

suppress adrenal functions, exogenous steroid may need to be administered, if patient is under stress (e.g. surgery and trauma).

Drug interactions

Microsomal enzyme inducers, if administered concomitantly may decrease medroxyprogesterone acetate activity. No other significant drug interaction has been reported with MPA or other progestogens.

Clinical uses

In veterinary medicine, MPA is used mainly to control oestrus and ovulation in cyclic ewes and to induce oestrus in anoestrus animals. It has also been used in bitches as a single injection in oil given in mid-anoestrus to suppress the onset of next oestrus. In cats, MPA has been used to treat sexually dimorphic behaviour problems such as roaming, inter-male aggressive behaviours, mounting and spraying. In canine aggression, the progestogens are believed to have an effect due to their anti-androgenic properties and also non-specific CNS depression. In humans, MPA is used parenterally as a long-lasting contraceptive in women, to decrease sexually deviant behaviour in men, and as an anti-neoplastic agent for some carcinomas (e.g. endometrial carcinoma and renal carcinoma).

Dose

For suppression/interruption of oestrus

Dogs (<15 kg body wt.) : 10 mg (total), PO, once daily for 4 days, then 5 mg daily for 12 days. It is given when pro-oestral bleeding is evident.

(>15 kg body wt.) : 20 mg (total), PO, once daily for 4 days, then 10 mg for 12 days.

Cats : 5 mg (total), PO, once daily given is dioestrus or anoestrus for long-term prevention of oestrus.

: 25 – 100 mg (total), IM or SC. It will inhibit oestrus from 2-4 months.

For prevention of oestrus

Dogs : 50 mg (total), SC, given once in anoestrus. Dose may be repeated after 3 – 6 months, if needed.

For synchronisation of oestrus

Sheep & goats : One sponge (60 mg), by intravaginal administration. The sponge is removed after 13-17 days.

For progesterone responsive dermatitis

Dogs : 20 mg/kg, IM, once daily. Dose may be repeated in 3-6 months, if needed.

Cats : 50 – 100 mg (total), SC or IM. Dose may be repeated in 3-6 months, if needed.

Megestrol acetate

Megestrol acetate (MA) is a synthetic progesterone derivative that is structurally and pharmacologically similar to medroxyprogesterone acetate. It has significant anti-estrogenic and corticosteroid activity with resultant adrenocortical suppression. Similar to medroxyprogesterone acetate, MA is well absorbed from the GI tract and appeared to slowly metabolised in the liver. The half-life of MA is reported to be about 8 days in dogs.

Side/Adverse effects of megestrol acetate are similar to those of medroxyprogesterone acetate. Prolonged administration of megestrol acetate may lead to profound adrenocortical suppression and adrenal atrophy with symptoms of adrenocortical insufficiency. Therefore, the MA should not be given for more than two consecutive treatments. Other side effects may include diarrhoea, rash and decreased libido.

In small animals, megestrol acetate is used to suppress or postpone oestrus. It is also used clinically for many dermatological and behaviour related conditions, particularly in the cat. In cats, eosinophilic granuloma and miliary dermatitis (crusting dermatosis) are responsive to MA because it has a glucocorticoid-like anti-inflammatory effect. However, some practitioners do not recommend megestrol acetate for the control of miliary dermatitis or eosinophilic granuloma complex due to unacceptable side effects of the drug. Therefore, potential risks versus benefits should be weighed before institution of MA therapy.

Dose

Prevention of oestrus

Dogs : 2 mg/kg, PO, once daily for 8 days given at pro-oestrus.

Cats : 5 mg (total), once daily for 3 consecutive days given in pro-oestrus.

Postponement of oestrus

Dogs : 0.5 mg/kg, PO, once daily for 32-40 days given in anoestrus and 7-14 days before postponement is required.

Cats : 2.5 mg/kg, PO, once weekly for up to 30 weeks given in anoestrus.

For treatment of idiopathic miliary dermatitis and eosinophilic granuloma.

Cats : 2.5 – 5 mg (total), PO, every 1-2 days until lesion regress, then once weekly until satisfactory response.

Melengestrol acetate

Melengestrol acetate (MGA) is another synthetic progestogen, which is structurally similar to endogenous hormone progesterone. It is used to suppress oestrus in cattle. Melengestrol acetate is also used as a growth promoter in cattle. It has been suggested that MGA primarily blocks the gonadotropins surge but does not affect the basal levels of gonadotropins in heifers. Hence treated animals develop large unovulated follicles which secrete more oestrogen, thereby causing increase in growth rate by 5-10% in treated heifers. For anabolic and growth promoting effect, MGA is used as a feed additive. It is also used occasionally to synchronize oestrus in heifers intended for breeding. Breeding age heifers are fed MGA daily for not more than 24 days and after the drug is removed heifers begin cycling more synchronously. A withdrawal period of at least 48 hours should be followed when using MGA in cattle.

Dose

Cattle : 0.25 – 0.5 mg/kg/day, PO in feed.

Hydroxyprogesterone

Hydroxyprogesterone (17-Hydroxyprogesterone, 17-OH progesterone, 17-OHP) is a 21-carbon natural progestogen produced during the synthesis of glucocorticoids and sex steroids (see Figure 48.2). This hormone is primarily produced in the

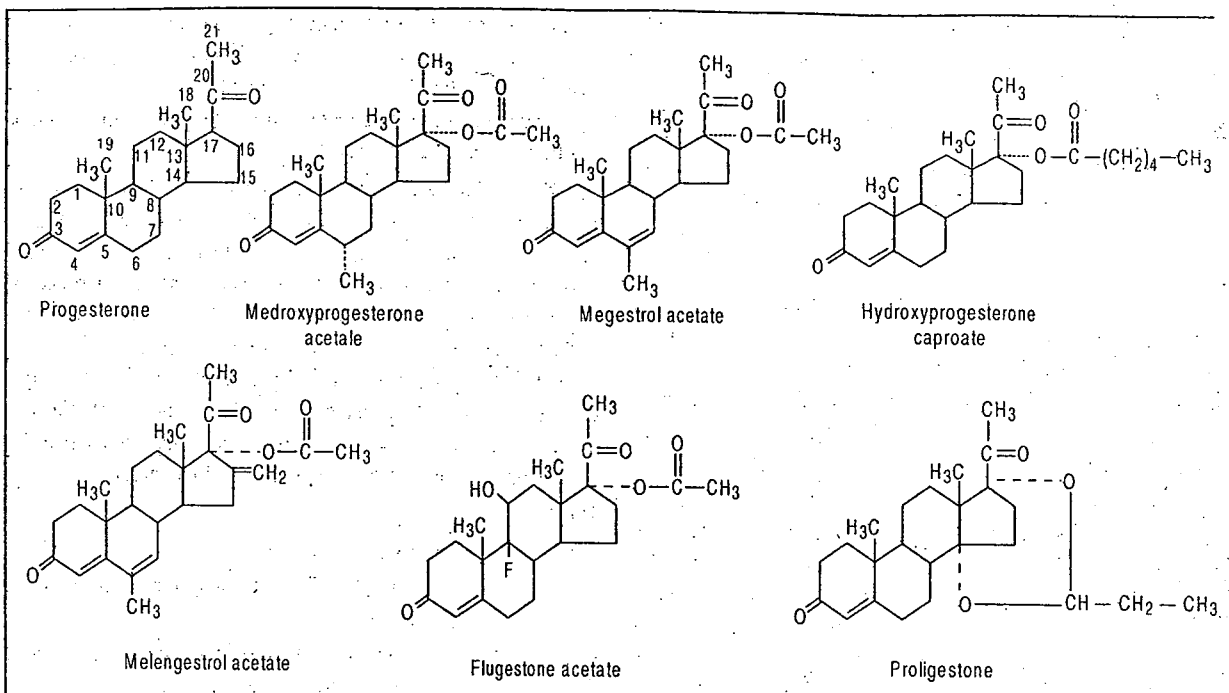


Fig. 52.6. Chemical structures of progesterone and selected progesterone derivative progestogens

adrenal glands and to some degree in the gonads, specifically the corpus luteum of ovary. Hydroxyprogesterone itself has poor progestational activity, but some of its esters like hydroxyprogesterone caproate (hydroxyprogesterone hexanoate) possess progestational activity. Esters of hydroxyprogesterone are occasionally used in the therapeutics and have actions similar to other progestogens. Hydroxyprogesterone caproate is used parenterally due to first-pass hepatic metabolism. It is available as an oily solution for intramuscular use and has long duration of action.

Flugestone

Flugestone acetate (Flurogestone acetate, flugesterone acetate, FGA) is a progestogen that is mainly administered as intravaginal sponge in ewe and doe to control or induce oestrus as described for progesterone. When the FGA impregnated sponge is used, the progestogen is absorbed and subjects the animal to a progestational action comparable to the luteal phase of the sexual cycle. This artificially imposed progestational phase is ended by the removal of the sponge. On withdrawal of sponge, gonadotropins may be administered as a single dose. Injection with equine chorionic gonadotropin (pregnant mare serum gonadotropin) at the time of sponge removal induces a simultaneous start of the follicular phase in the treated animals. Follicles then develop and synchronised oestrous (oestrus) and ovulation follow. Milk taken from animals during sponge insertion and within 14 days after removal must not be used for human consumption.

Dose

For synchronisation of oestrus/induction of oestrus and ovulation during non-breading season

Sheep & goats : 1 sponge/pessary (30 mg FGA) by intravaginal administration. Sponge is removed after 12-14 days. It may be followed within 6 hours in non-breading season by serum gonadotropins (500 units, SC or IM).

Proligestone

Proligestone is a week progestogen with antigonadotrop activity. It is used mainly to suppress or postpone oestrus in bitches. It can be given at intervals of 3-5 months and is effecti regardless of stage of cycle when given. Side effects relating to uterine disorders, mammary tumours and behavioural chang were reported to be very low in treated animals. It is administered by SC injection in the form of an aqueous suspension.

Dose

For postponement of oestrus

Dogs : 10 – 30 mg/kg, SC. Dose may be repeated intervals of 3 to 5 months.

Cats : 100 mg (total), SC.

Other progesterone derivatives

Several other synthetic analogues of progesterone are available commercially and are used occasionally in veterina and/or human medicine. These include chlormadinone, dydrogesterone and medrogestone. The chlormadinone acetat similar to delmadinone acetate, possesses significant anti-androgenic activity. Dydrogesterone is a potent, orally activ progestogen indicated in a wide variety of gynaecological conditions. Medrogestone, a synthetic drug with similar effec as progesterone, is occasionally used in the treatment of endometrial cancer, amenorrhea and in some regimens for brea cancer in women and benign prostatic hyperplasia in men.

3. 19-Norprogesterone Derivatives

The 19-Norprogesterone is structurally similar to th endogenous progesterone, except that C-19 methyl group of progesterone is replaced by a hydrogen atom. Some derivative of 19-norprogesterone possess progestational activity and are used clinically in several gynaecological disorders. The spectrum of activities of these compounds is highly dependent on specific subconstituent group. These drugs are commonly used in human medicine and have limited application in veterinary practice.

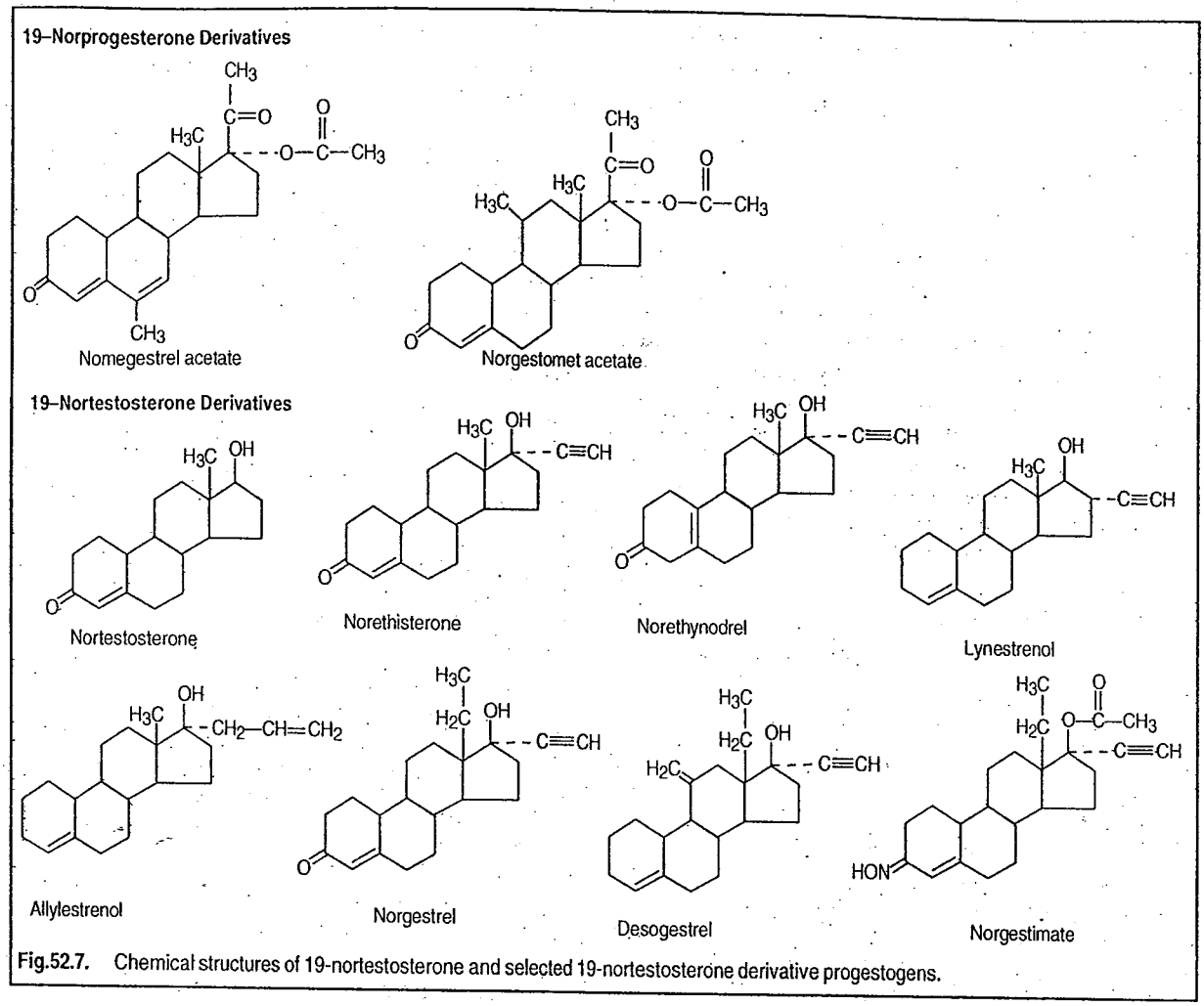


Fig.52.7. Chemical structures of 19-nortestosterone and selected 19-nortestosterone derivative progestogens.

Nomegestrol

Nomegestrol acetate is a potent, orally active 19-norprogesterone derivative progestogen. It binds specifically to the progesterone receptor and relatively lacks affinity for other steroid receptors. Nomegestrol acetate has potent antigonadotropic activity and exerts strong anti-oestrogenic effects at the level of the endometrium, but without any residual androgenic or glucocorticoid properties. The antigonadotropic action of nomegestrol acetate is mediated, like other progestogens, at the hypothalamic and pituitary level. Moreover, nomegestrol acetate has partial antiandrogenic activity. Nomegestrol acetate has been used in human patients for the treatment of some gynaecological disorders such as menstrual disturbances, dysmenorrhoea and premenstrual syndrome. It is also a component of hormonal replacement therapy in combination with estradiol for the relief of menopausal symptoms.

Norgestomet

Norgestomet is a synthetic 19-norprogesterone progesterone that is used mainly in combination with estradiol valerate to synchronise oestrus in dairy cattle. Norgestomet is administered to heifers or cows in a special delivery system (hydron) ear implants to control or induce ovulation.

Other drugs

Other 19-norprogesterone derivative progestogens mainly used in human medicine include trimegestone, gestonorone and promegestone. Trimegestone, a novel 19-norprogesterone progestogen, is being used in combination with estradiol in human practice for the treatment of menopausal symptoms.

4. Testosterone or 19-Nortestosterone derivatives

Testosterone or 19-Nortestosterone derivatives are 18-carbon steroid progestogens which lack C-19, C-20, and C-21 carbons found in the progesterone and hence resemble the testosterone. The 19-Nortestosterone (similar to testosterone except that C-19 methyl group is replaced by a hydrogen atom) itself is inactive, but some of its derivatives have potent progestational activity and also have a variety of androgenic, glucocorticoid and other activities. Ethisterone (17α-ethynyltestosterone), a testosterone analogue, was the first orally active progestogen marketed for use in humans in early 1940'. Subsequently a large number of 19-nortestosterone derivative progestogens were synthesised and used in human medicine.

The spectrum of activities of 19-Nortestosterone derivatives is highly dependent on specific subconstituent group. An ethinyl substituent at C-17 decreases hepatic metabolism and yields 19-nortestosterone progestogens such as norethisterone (norethindrone), norethynodrel, ethynodiol diacetate, lynestrenol (ethinylestrenol) and allylestrenol, which are orally active. Some newer compounds (also older norgestrel) like desogestrel, norgestimate, gestodene and etonogestrel which have ethyl group in place of methyl group at C-13 (13 ethyl substitutions) are called gonanes. The gonanes are reported to have minimal of androgenic activity.

The 19-nortestosterone derivative progestogens are commonly used in human medicine with oestrogens (combination/ sequential preparations) for oral contraception in women. These compounds, however, have no/limited place in veterinary therapeutics.

5. Miscellaneous Progestogens

Altrenogest

Altrenogest, also be known as allyl trenbolone, is an orally administered synthetic progestational agent. It is used in mares, gilts and sows to synchronise or suppresses oestrus and extend luteal phase. In mares, oestrus generally ensues 2-5 days after treatment is completed and most animals ovulate between 8-15 days after withdrawal. Its effectiveness to suppress oestrus is used in suppressing oestrus expression in exhibition or race mores. Its side/ adverse effects are similar to other progestogens. Altrenogest is usually administered in feed. Partly consumed medicated feed should be safely destroyed and not given to any other animal

Dose

For suppression of oestrus

Horses : 0.04 mg/kg, PO, daily for 10-15 days.

For synchronisation of oestrus

Pigs (gilts) : 20 mg, PO, daily for 18 days.

(sows) : 20 mg, PO, daily for 3 days.

Other agents

Cyproterone, tibolone, drospirenone, norelgestromin and quingestanol are some other synthetic progestogens available for use in human medicine. Cyproterone acetate is a synthetic derivative of 17-hydroxyprogesterone and acts as an androgen receptor antagonist with weak progestational and glucocorticoid activity. Tibolone, a synthetic steroid that binds non-selectively to all nuclear receptors, is used mainly for treatment of endometriosis as well as hormone replacement therapy in post-menopausal women. Drospirenone is a synthetic progestin and an analogue to spironolactone. Norelgestromin (also known as deacetylnorgestimate) has high progestational, slight oestrogenic and low androgenic activity; it is mainly used as a hormonal contraceptive. Quingestanol has actions similar to those of progesterone.

II. Drugs used in Excess of Progestogens/ Antiprogestogens

Antiprogestogens (Antiprogestins) are drugs which inhibit the biological action of progesterone and synthetic progestogens. The concept of antiprogestogens is comparatively new with limited number of drugs available for therapeutic use. In veterinary medicine, these drugs have been used experimentally and therapeutically to terminate pregnancy in dogs.

1. Selective Progesterone Receptor Antagonists

Mifepristone

Mifepristone is a derivative of 19-nortestosterone norethisterone (norethindrone). It possesses potent antiprogestational activity. Studies have indicated the effectiveness of mifepristone in dogs. On progesterone receptor, it acts as a partial agonist i.e. in presence of progestogens it acts as a competitive receptor antagonist, but alone it shows weak progestational activity.

Mechanism of action

Mifepristone is a competitive antagonist of progesterone binding to its specific progesterone receptor. In the presence of progesterone, mifepristone acts as a competitive progesterone receptor antagonist, but in absence of progesterone it acts as a partial agonist. In addition to being an antiprogestogen, mifepristone is also an antiglucocorticoid and a weak antiandrogen. Mifepristone's relative binding affinity at the progesterone receptor is more than twice that of progesterone, its relative binding affinity at the glucocorticoid receptor is more than ten times that of hydrocortisone; and its relative binding affinity at the androgen receptor is less than one third that of testosterone. It has no action at oestrogen receptor or mineralocorticoid receptor.

Pharmacological effects

Administration of mifepristone to females early in pregnancy may result in abortion of foetus due to interference with endogenous progesterone causing detachment of the blastocyst. Mifepristone also sensitises uterus to the contractile actions of prostaglandins (simultaneous administration of $PGF_{2\alpha}$ enhances its abortifacient effect). Given during the luteal phase of oestrus cycle, it prevents uterine glandular secretory changes normally brought about by progesterone and may accelerate degenerative and vascular changes leading to recycle.

The antiglucocorticoid action of mifepristone is usually not observed at normal doses because inhibition of negative feedback at pituitary-hypothalamic levels elicits CRH and/or ACTH release, thus enhancing hydrocortisone secretion in the plasma. The enhanced circulating adrenal steroid levels in plasma overcome the direct competitive anti-glucocorticoid action of the drug.

Pharmacokinetics

Pharmacokinetics data on mifepristone in domestic animals are limited. In man, mifepristone is active orally, but its bioavailability is only 25%. It is largely metabolised in liver and excreted in bile. Plasma half-life of mifepristone is about 20 to 40 hours in human beings.

Side/Adverse effects

Detailed studies on the long-term effects of mifepristone in humans or animals have not been documented. Some studies on experimental animals have revealed the teratogenic potential of mifepristone in rabbits, but not in rats and mice.

Contraindications and precautions

Mifepristone is contraindicated in patients with ectopic pregnancy, adrenal failure, hemorrhagic disorders, inherited porphyria and anticoagulant or long-term corticosteroid therapy.

Clinical uses

Mifepristone is used mainly in human medicine to produce medical abortion in the first trimester of pregnancy. Administration of prostaglandin E_1 intravaginally or misoprostol (an analogue of PGE_1) orally after a single oral dose of mifepristone effectively terminates gestation in women. In humans, mifepristone is also used as a contraceptive given during the luteal phase of the cycle. Preliminary studies have established the efficacy of mifepristone as an abortifacient in bitches. However, further studies are required to establish its effectiveness in animals.

Onapristone

Onapristone is a mifepristone analogue with antiprogestin activity but with lowered antiglucocorticoid action. It has demonstrated therapeutic potential for the treatment of hormone dependent breast cancer.

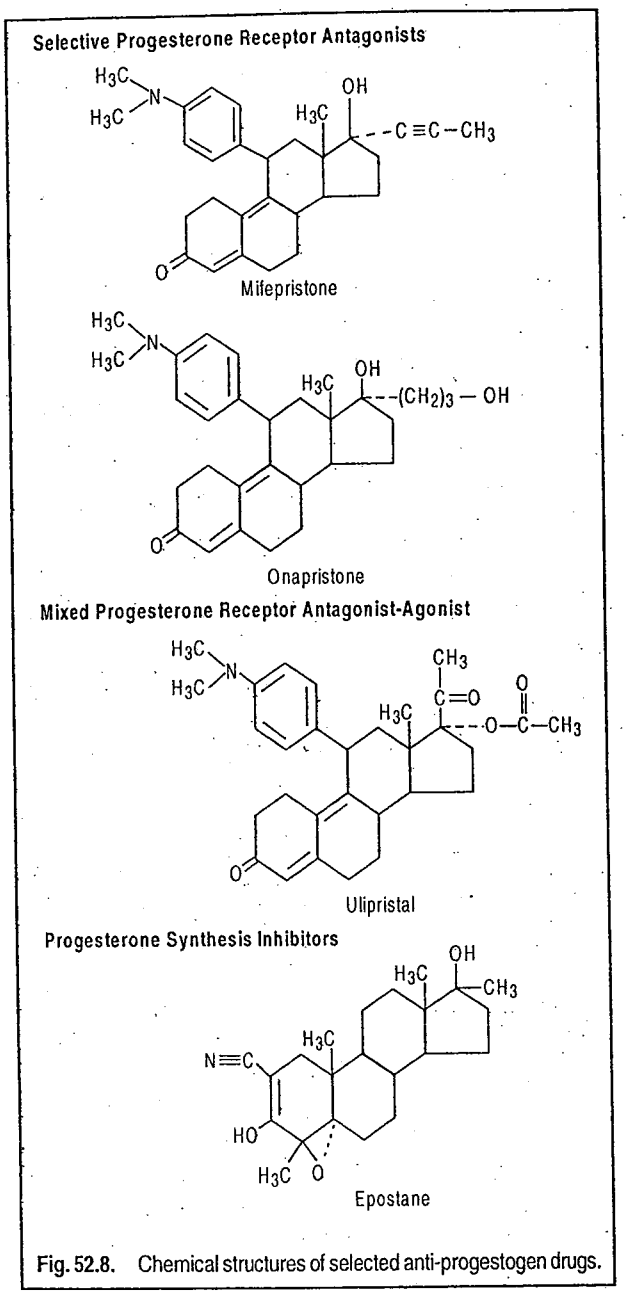


Fig. 52.8. Chemical structures of selected anti-progestogen drugs.

2. Mixed Progesterone Receptor Antagonist-Agonists/ Selective Progesterone Receptor Modulators

Ulipristal

Ulipristal acetate is a newly introduced selective progesterone receptor modulator (SPRM) with antagonistic as well as partial agonistic effects on the progesterone receptor. It also binds to the glucocorticoid receptor, but has no relevant affinity to the oestrogen, androgen and mineralocorticoid receptors. Some studies have shown that ulipristal helps in blocking or delaying the ovulation and in delaying the maturation of endometrium. Ulipristal acetate has been approved for humans as an emergency contraception within 5 days after an unprotected intercourse. Common side effects include abdominal pain and menstrual disorder.

Asoprisnil

Asoprisnil is another selective progesterone receptor modulator (SPRM) with effects similar to those of ulipristal acetate. It is an investigational drug.

2. Progesterone Synthesis Inhibitors

Epostane

Epostane is a progesterone synthesis inhibitor. It acts as a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, an enzyme that catalyses the synthesis of progesterone from pregnenolone i.e. 17-hydroxyprogesterone from 17-hydroxypregnenolone, and androstenedione from dehydroepiandrosterone in the adrenal gland. After 7 days of epostane treatment, the synthesis of progesterone stops and its concentration declines to levels which do not support corpus leutem. This results in the death of foetus. Epostane has been used as an antiprogesterone agent in veterinary practice to terminate pregnancy in bitches and to induce farrowing in sows. The side/adverse effects reported with epostane are minimal.

MALE GONADS

The male gonads or testes are the primary organs of reproduction in the male. Similar to the ovaries, testes are components of both the reproductive system and the endocrine system. The primary functions of testes are to produce sperm (gametogenesis) and to produce androgens (steroidogenesis), primarily testosterone. Both functions of testes are influenced by gonadotropic hormones produced by the anterior pituitary.

ANDROGENS

The testes secrete several male sex hormones which are collectively called androgens. These include testosterone, dihydrotestosterone, androstenedione and dehydroepiandrosterone. Testosterone is the main androgen. Androgens are produced mainly by the interstitial cells of the testes and to a lesser extent by the adrenal cortex, ovaries and also epididymis in the stallion. The androgens are mainly concerned with spermatogenesis, growth, development of accessory sex organs and secondary sexual characteristics in the male. In addition, they have marked anabolic effects in puberty causing development of the musculature and increased bone growth. The testes in addition to androgens also synthesise small amounts of oestrogens, which are probably also essential for spermatogenesis.

Synthesis, Release, Transport and Fate

Synthesis and release: All androgens are steroidal compounds which are synthesised mainly from cholesterol and also from acetyl coenzyme A. The synthesis of testosterone and some minor androgens utilise the pathway depicted in Figure 48.2. Dihydrotestosterone is produced mainly as an active metabolic product of testosterone. The relative potency of testosterone, 5-α-dihydrotestone, androstenedione and dehydroepiandrosterone are 100, 200, 16 and 8, respectively.

The synthesis and release of androgens, in particular testosterone, are controlled largely by luteinising hormone (LH), that in male is also called interstitial cell stimulating hormone (ICSH). The interstitial cells of Leydig, which synthesise testosterone, also have receptors for prolactin that may influence testosterone production by increasing the number of receptors for ICSH. Growth hormone may have a synergistic effect with LH on testes, while oestrogens can decrease effects of LH on secretion of testosterone. Adrenal production of androgens is under the influence of corticotropin.

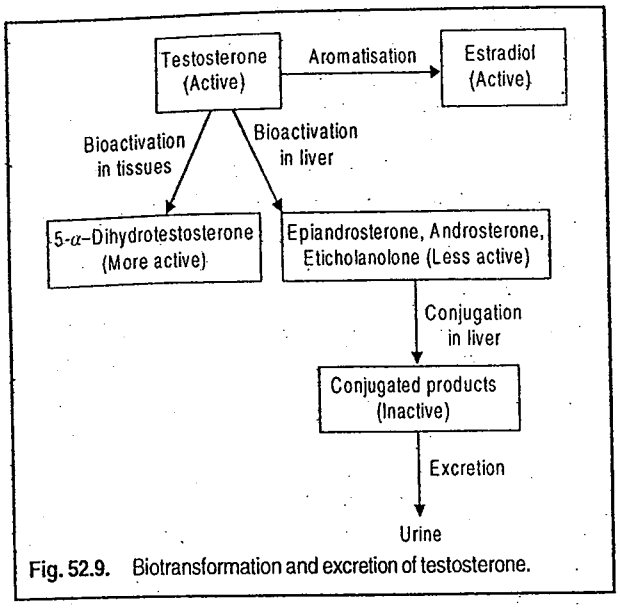


Fig. 52.9. Biotransformation and excretion of testosterone.

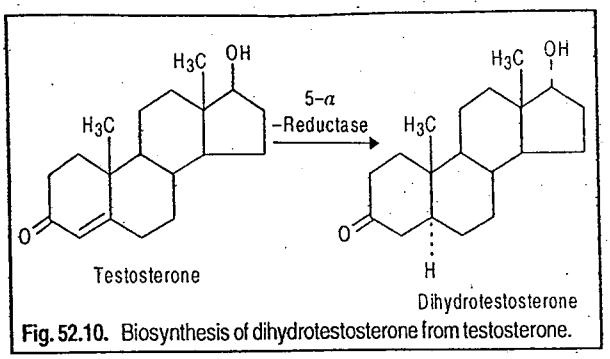


Fig. 52.10. Biosynthesis of dihydrotestosterone from testosterone.

Transport and fate: After secretion by the testes, most of testosterone (~97%) becomes bound with a beta globulin called sex-hormone binding globulin or plasma albumin and circulates in blood. In peripheral tissues, most of the testosterone is converted within cells to dihydrotestosterone, which is the active intracellular androgen. Some actions of testosterone are dependent on this bioactivation conversion, while other actions are not. The testosterone that does not enter tissues is mainly inactivated in the liver to androsterone, epiandrosterone and eticholanolone which in turn are conjugated with sulphate and glucuronic acid (Figure 52.9) and excreted either into gut or into urine. Dihydrotestosterone itself is converted in the liver to androsterone, androstanedione and androstanediol. Minor androgens like dehydroepiandrosterone and androstenedione, which may be released from the gonads and the adrenal cortex in both sexes, are subsequently converted to testosterone in the liver. Testosterone also can aromatised to estradiol in a variety of extraglandular tissues, a pathway that accounts for most oestrogen synthesis in males.

Mechanism of Action

Like other steroid hormones, testosterone in lipophilic and hence diffuse rapidly across the cell membrane in target cells. Although at some sites (e.g. hypothalamus/pituitary, spermatogenic cells in testes, muscles and liver) testosterone itself is active, at most target tissues such as prostrate, epididymis, seminal vesicles, skin and liver testosterone is converted to the more active dihydrotestosterone by the enzyme steroid 5α-reductase (Figure 52.10). The 5α-reductase enzyme exists in two isomers viz. 1 and 2, with different distribution in body. Steroid 5α-reductase 1 is located largely in the non-genital skin and liver, and steroid 5α-reductase 2 is present in the urogenital tract of male and in the genital skin of both sexes. Testosterone or dihydrotestosterone then binds to an intracellular receptor protein called androgen receptor (AR) and modify gene transcription by the same mechanism as other steroid hormones (Figure 52.11). Androgen regulated genes are critical for the development and maintenance of the male sexual phenotype.

Physiological effects

The androgens in general are responsible for the development of primary and secondary sex characters in males and are necessary for spermatogenesis.

Sex organs: The androgens are responsible for the growth and development of male gonads. The androgens secretion causes penis, scrotum and testes to enlarge several fold after puberty. During embryonic life, androgens virilise the urogenital tract of the male embryo and are responsible for development of male phenotypes. Lack of androgens in male foetus usually results in the development of female external phenotype. Androgens are responsible for descend of testis into the scrotum in male foetus. Androgens are required for the spermatogenesis in seminiferous tubules and for the maturation of sperm in passage through epididymis and vas deferens.

Secondary sex characteristics: Androgens are responsible for development of secondary sex characteristics in males. They influence hair pigmentation and growth in birds. The hair coat of males tends to be darker, especially in certain body regions than the females. Androgens maintain the normal libido in males. Production and release of pheromones by males of some species also depend on the androgens. In humans, androgens are responsible for growth of hair on the pubic, axillary, chest and face. Androgens also may be responsible in part for the aggressive and sexual behaviour of males in some species of animals.

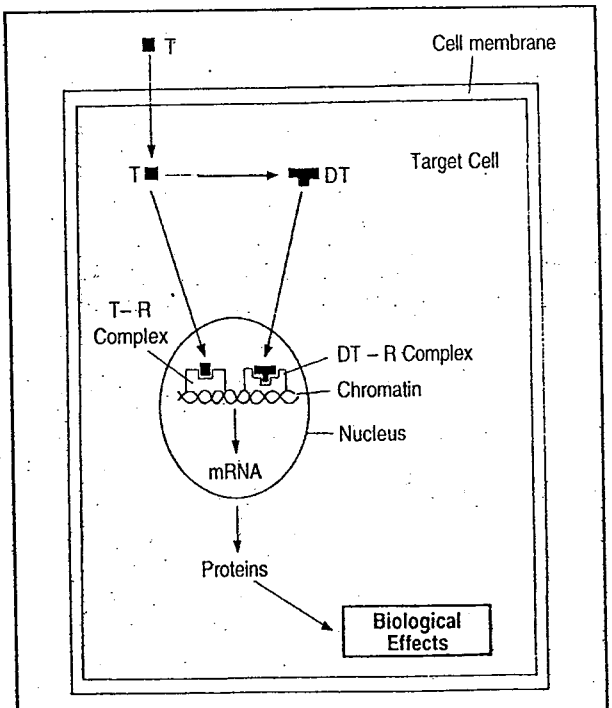


Fig. 52.11. Schematic representation of mechanism of action of androgens.

Abbreviations : T=testosterone ; DT = dihydrotestosterone ; R=receptor ; mRNA = messenger ribonucleic acid.

Metabolic effects: Androgens have anabolic effects and they favour growth of skeleton and musculature with increase in body weight. They cause increased protein synthesis, particularly in the skeletal muscle, mainly by their nitrogen retaining effect and also through stimulation of certain protein synthesising enzymes. Androgens favour calcium retention and cause growth of bones, cartilages and other tissues. Like estradiol, testosterone is also responsible for closing of epiphyseal plates. They also retain potassium, sodium, chloride, phosphate and sulphur and hold water in association with the retained electrolytes. However, the electrolyte and water retention occurs only to a minor degree under physiological conditions. Androgens accelerate erythropoiesis by increasing erythropoietin production and probably by direct action on haem synthesis.

Hypothalamic-pituitary-gonadal axis: Androgens produce a negative feedback effect on hypothalamus and/or pituitary and modulate the concentration of gonadotropins in circulation. While relatively high concentration of testosterone inhibits LH (ICSH) secretion, it has only weak inhibitory action on FSH secretion. Oestrogens are reported to be more potent inhibitors of gonadotropins secretion even in males and it believed that the small amounts of estradiol produced by testes and that resulting from conversion of testosterone to estradiol play a role in feedback inhibition.

DISORDERS OF ANDROGENS

Deficiency of Androgens

Deficiency of androgens, a condition called hypoandrogenism, may result from hypogonadism in males. This deficiency may also be caused by problems of the pituitary gland or hypothalamus. In some cases, androgens may be present in sufficient concentrations, but there is genetic absence of androgens receptors in the target cells. The effects of androgen deficiency depend on severity of deficiency, its cause and the age at which the deficiency begins. When testes are non-functional during foetal life, the male sexual organs do not develop, instead female organs are formed. If testes fail to function or are removed before puberty, normal pubertal changes do not occur and sexual organs and secondary sexual characteristics remain those of a young animal and there is no libido. After puberty, sexual organs regress slightly in size and libido may be lost. Failure of testes to develop may result from a deficiency of gonadotropins or from a primary testicular defect. Complete failure of the endocrine function of the testis in adult is not common; a partial deficiency can originate from incomplete testicular development at puberty or from a disorder during adult life such as pituitary failure or a viral infection of the testis.

Hypogonadism is a rare condition in animals.

Excess of Androgens

Hypersecretion of androgens by testes, a condition called hyperandrogenism, occur rarely in animals. A rare interstitial Leydig cell tumour may sometimes produce as much as 100 times the normal quantity of testosterone. Such interstitial cell tumours cause excessive development of male sexual organs in males and clitoris in females, all muscles and other secondary male sexual characteristics. If it occurs before puberty, excessive androgen secretion may cause rapid growth of the musculature and bones, but also cause early uniting of the epiphyses. An occasional adrenocortical tumour, reported mainly in humans, also secrete more quantities of androgens, which may cause excessive masculinising effects.

TREATMENT OF ANDROGEN DISORDERS

Disorders of androgens can be treated by using a wide variety of drugs possessing either testosterone-like androgenic and/or anabolic activity, or androgen suppressing activity. These drugs are commonly used in human medicine, but have limited clinical use in animals.

Classification

Drugs used to treat androgen disorders have been broadly classified into two classes: drugs used in deficiency of androgens and drugs used in excess of androgens.

I. Drugs used in deficiency of androgens
  A. Androgenic steroids/Drugs producing primarily androgenic effects
    1. Androgen receptor agonists
    a. Natural androgenic steroids
      e.g. testosterone and androstanolone (dihydrotestosterone).
    b. Synthetic androgenic steroids
    i. Synthetic esters of testosterone
      e.g. testosterone cypionate, testosterone propionate, testosterone enanthate, testosterone decanoate and testosterone phenylpropionate.
    ii. Synthetic analogues of testosterone
      e.g. methyltestosterone, mibolerone, danazol and mesterolone.
    2. Selective androgen receptor modulators
      e.g. andarine and ostarine.
  B. Anabolic steroids/Drugs producing primarily anabolic effects
    1. Androstane anabolic steroid /Testosterone derivatives
      e.g. stanozolol, boldenone, fluoxymesterone, methandrostenolone methandriol, oxandrolone and oxymetholone.
    2. Estrene anabolic steroids /Nor-testosterone derivatives
      e.g. nandrolone, trenbolone, ethylestrenol, norethandrolone and bolenol.
    3. Prohormones of anabolic steroids
      e.g. 4-androstenedione, 4-androstenediol, 19-norandrostenedione and 1-testosterone.

II. Drugs used in excess of androgens
  1. Inhibitors of androgen synthesis
  a. 5α-Reductase inhibitors
    e.g. finasteride and dutasteride.
  b. CYP17A1 inhibitors
    e.g. abiraterone.
  c. Gonadotropin-releasing hormone and synthetic analogues
    e.g. gonadorelin and leuproline.
  d. Miscellaneous agents
    e.g. ketoconazole and spironolactone.
  2. Androgen receptor antagonists
    e.g. cyproterone acetate, flutamide, bicalutamide and nilutamide.
  3. Female sex hormones
    e.g. delmadinone and dienogest.

I. Drugs Used in Deficiency of Androgens

The androgens are a group of endogenous steroids which produce or stimulate the development of male characteristics. Conventionally, the term 'androgens' include natural androgens as well as their synthetic analogues which have masculinising and/or anabolic effects. Treatment of androgen deficiency in males involves primarily testosterone replacement therapy.

A. Androgenic Steroids / Drugs Possessing Primarily Androgenic (Masculinisation) Effects

1. Androgen Receptor Agonists

a. Natural Androgenic Steroids

Testosterone

Testosterone is natural androgen that is secreted primarily by the testes. Free testosterone is available commercially but is less used in therapeutics due to its short half-life. As with other sex steroids, testosterone is ineffective orally because of rapid inactivation by first-pass metabolism. Testosterone injection (IM) as a solution in oil usually maintains a circulating level of hormone for 1-3 days. However, it still has shorter duration than its synthetic ester compounds. Implants of the hormone may maintain a level for 1-2 weeks.

Dose

Dogs : 5 mg (total), IM, 2 to 3 times per week.

Androstanolone

Androstanolone is the drug form of the endogenous androgen dihydrotestosterone, which is formed by the peripheral conversion of testosterone. Androstanolone acts on the androgen receptor and has a predominantly androgenic effect. Androstanolone was widely abused by athletes; therefore it is now no longer commercially available.

b. Synthetic Androgenic Steroids

i. Synthetic Esters of Testosterone

Testosterone esters such as testosterone-cypionate, (testosterone cyclopentyl-propionate), -propionate, -phenylpropionate, -enanthate (-heptanoate), -decanoate and -isocaproate are available commercially and are more widely used than the free testosterone. The ester chain is typically attached to the parent steroid hormone at the 17th carbon position (beta orientation), although some compounds carry esters at position 3. The synthetic esters of the testosterone determine the solubility of the compound and also the duration of action. These esters are administered intramuscularly and are clinically used to promote and maintain primary and secondary male sexual characteristics. These drugs may also be used for suppression of oestrus in females.

Pharmacological effects

The mechanism of action and pharmacological effects of testosterone esters are essentially similar to those of physiological effects of testosterone discussed earlier. Testosterone released slowly from the ester promotes and maintains masculinisation and increases libido. Although testosterone esters are used in the treatment of deficient libido in males, they actually can induce infertility by negative inhibition on LH and FSH release, which are necessary for spermatogenesis. Testosterone esters have anabolic activity with resultant increased protein anabolism and decreased protein catabolism. Testosterone and its esters demonstrate a 1 : 1 ratio of androgenic to anabolic activity.

Pharmacokinetics

Most esters of testosterone must be given by injection because they undergo hepatic first pass metabolism. Testosterones esters are less polar and more soluble in the lipid vehicle used for injection hence are absorbed more slowly when given by

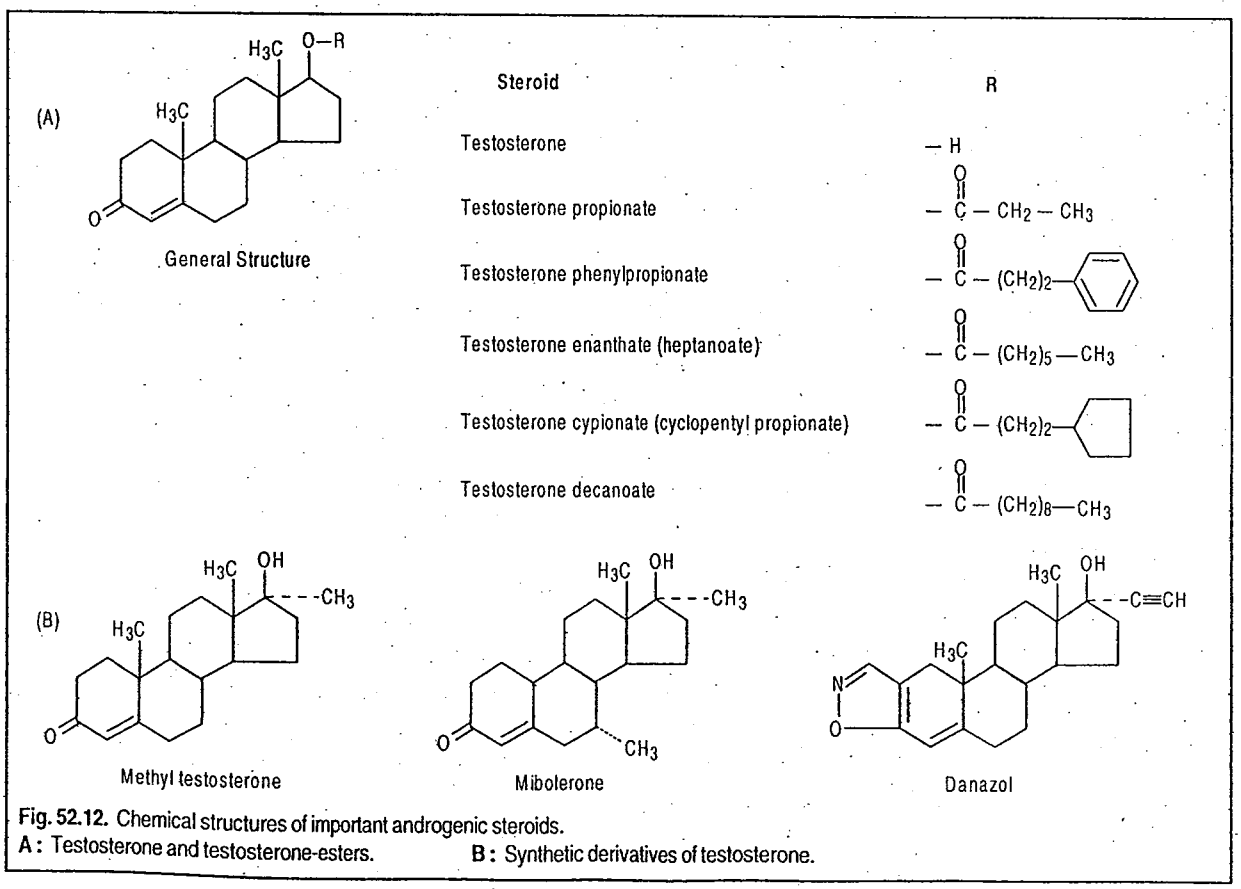


Fig. 52.12. Chemical structures of important androgenic steroids.
A: Testosterone and testosterone-esters. B: Synthetic derivatives of testosterone.

intramuscular route. In general, the release of active hormone depends on the length of the carbon chain i.e. the longer the carbon chain in the ester, the more slowly the testosterone is released from the vehicle. Therefore, testosterone propionate has shorter duration of action than the enanthate, cypionate or decanoate ester.

The free testosterone after its release from ester group binds (~98%) to a specific sex-hormone binding globulin. It is metabolised in the liver to less active/inactive metabolites as discussed earlier. The plasma half-life of testosterone depends on the type of ester group attach to it. The half-life of free testosterone is 10-20 minutes but that of testosterone cypionate is about 8 days. The duration of action of testosterone cypionate may persist for 2-4 weeks after IM injection.

Side effects/Adverse effects

Testosterone therapy may lead to oligospermia or infertility in males. Perianal adenomas, perineal hernias, prostatic disorders and behavioural changes are possible following long-term testosterone treatment. Masculinisation of the female may occur, particularly after long-term treatment and/or overdosage. Testosterone has sodium-retaining activity, which can lead to water retention, oedema formation and gain in weight. Chronic administration of testosterone may cause testicular degeneration of male animals.

Contraindications and precautions

Testosterone is contraindicated in pregnant animals, hepatic impairment and congestive heart failure. Use of androgens during pregnancy may lead to masculinisation of female foetuses. Testosterone therapy should not be given to animals suffering from conditions known to be aggravated by androgens such as prostatic hypertrophy or androgen dependent neoplasia. It should be used with caution in patients with renal dysfunction.

Drug interactions

Testosterone may enhance the fluid retention and oedema formation when given along with corticosteroids. Testosterone may cause increased bleeding when administered concurrently with oral anticoagulants. Testosterone may decrease blood glucose levels and hence insulin requirements.

Clinical uses

Testosterone preparations are infrequently used to control impotency and infertility in male animals mainly due to their variable effects. In veterinary medicine, testosterone is used primarily for the treatment of testosterone responsive urinary incontinence and hormonal alopecia in neutered male dogs. Testosterone preparations are also useful to suppress ovarian activity and thereby prevent oestrus cycle in bitches. However, persistent anoestrus is seen in greyhound bitches treated with testosterone to prevent oestrus during racing. Occasionally, testosterone is used for mammary tumours and pseudopregnancy with lactation in bitches. Testosterone preparations are also used in bovine medicine to produce a teaser animal (oestrus detector) in cull cows, heifers and steers. Testosterone propionate in combination with estradiol benzoate in the form of compressed tablet implant is used as growth promoter in farm animals (see Chapter 75).

Dose

For testosterone-responsive urinary incontinence

Testosterone cypionate

Dogs : 200 mg (total), IM once per month.

Testosterone enanthate

Dogs : 1 – 2 mg/kg, IM, once every 2 to 4 weeks. Maximum dose at one injection should not exceed 30 mg.

Testosterone propionate

Dogs : 1 – 2 mg/kg, IM or SC, 2 to 3 times every week.

Cats : 5 – 10 mg (total), IM.

To produce teaser animal

Testosterone propionate

Cattle : 200 mg (total), IM on day 1 and on days 4-9; on day 10 one gram IM should be given. To maintain teaser, booster dose may given at rate of 1 gram every 10-14 days.

b. Synthetic Analogues of Testosterone / Oral Androgens

Several synthetic analogues of testosterone are available commercially for oral dosing. Alkylation at the 17α-position considerably increases resistance of the compound to inactivation by liver and ensures high concentrations of the drug in systemic circulation. Alkylation also increases the hepatotoxicity of any given steroid.

Methyltestosterone

Methyltestosterone (17α-Methyl testosterone) is a synthetic derivative of testosterone in which a methyl group has been added at C-17 position. Methyltestosterone is unique among 17α-substituted alkyl derivatives in retaining androgenic potency while at the same time being active orally.

Pharmacological effects

The mechanism of action and pharmacological effects of methyltestosterone are similar to those of testosterone. However unlike testosterone esters, the methyl group itself is not removed metabolically and hence the alkylated hormone mediates the action within cells. Methyltestosterone is less efficacious and less potent than parenteral testosterone esters.

Pharmacokinetics

Methyltestosterone is well absorbed after oral administration in simple stomached animals. It is slowly metabolised in liver and hence maintains the required concentration in systemic circulation. The effects of methyltestosterone usually last for 1 to 3 days.

Side effects/Adverse effects

Side/Adverse effects of methyltestosterone are similar to those produced by testosterone preparations. Methyltestosterone and other 17-alkyl substituted derivatives are reported to cause cholestatic jaundice in a dose-dependent manner, which is normally not observed with parenteral testosterone esters. Virilisation with overdosage and masculinisation in females may be seen after longer period of methyltestosterone therapy. Pregnant animals, hepatic impairment and congestive heart failure are absolute contraindications of methyltestosterone. Abrupt withdrawal of drug following long-term administration should be avoided.

Clinical uses

Methyltestosterone is used clinically for the treatment of oestrogen-dependent mammary tumours, suppression of oestrus, pseudopregnancy and certain hormonal alopecia in dogs. In male dogs, it is used occasionally to enhance libido but chances of infertility due to inhibition of gonadotropins release exist, especially after prolonged use.

Dose

Dogs & cats : 0.5 – 2 mg/kg, PO, once daily. Maximum dose should not exceed 30 mg/day.

Mibolerone

Mibolerone (Dimethyl nortestosterone) is a 19-nortestosterone derivative that possesses significant androgenic, anabolic and anti-gonadotropic activities. Mibolerone was first developed in 1963 and marketed as a veterinary pharmaceutical. When compared to testosterone, it is approximately 6 times more potent as an anabolic, and about 2.5 times more potent as an androgenic. Although it is a potent anabolic steroid, its use is largely approved as an androgenic compound to suppress estrous in adult female dogs.

Pharmacological effects

Like endogenous testosterone, mibolerone possesses androgenic, anabolic and anti-gonadotropic activities in animals. Although it is structurally related to 19-nortestosterone progestogens, mibolerone does not possess significant progestational activity in animals. Mibolerone selectively blocks the luteinizing hormone (LH) peak thereby preventing estrous activity. Because of lack of LH, follicles develop to a certain point, but do not mature and hence no ovulation or corpus luteum development occurs. This leads to suppression of ovarian activity and hence prevention of oestrus cycle. The duration of time from discontinuing administration to occurrence of next oestrus cycle is variable (7-200 days).

Pharmacokinetics

Like other 17α-alkylated androgens, mibolerone is well absorbed from the GI tract after oral administration and metabolised slowly in the liver. It is reported to be metabolised to at least 10 separate metabolites, which are excreted both in urine and faeces.

Side effects/Adverse effects

Side/Adverse effects of mibolerone are similar to those of testosterone preparations discussed earlier. Masculinisation of female, vulvo-vaginitis, abnormal behaviour, etc. have been observed following mibolerone therapy. Hepatic dysfunctions, renal toxicity and thyroid dysfunction may be observed in some animals. Persistent anoestrus (i.e. lack to return to oestrus cycle) is seen in some bitches. Immature females are more prone to adverse effects of mibolerone than the mature females.

Contraindications and precautions

Contraindications of mibolerone therapy are similar to those of testosterone. Mibolerone may inhibit lactation; therefore it should not be used in nursing bitches. Mibolerone is generally not used in cats because it has low margin of safety in this species.

Clinical uses

Mibolerone is specifically approved for prevention of oestrus (heat) in the bitches. It prevents oestral activities and hence is used as a contraceptive in bitches. Occasionally, it is used for the treatment of severe galactorrhoea and pseudopregnancy in bitches. Mibolerone is not approved for use in humans, but has found wide usage in bodybuilding community.

Dose

For suppression of oestrus

Dogs : 0.002 – 0.004 mg/kg, PO, once daily. Treatment should begin at least 30 days prior to proestrus.

For false pregnancy

Dogs : 0.016 mg/kg, PO, once daily for 5 days.

Danazol

Danazol is a synthetic derivative of ethisterone (ethinyl testosterone) that has ethinyl (ethynyl) group at C-17 position. Danazol is classified as an androgen, but it is reported to have multiple effects in the body. It possesses weak androgenic, anabolic, anti-oestrogenic and progestational activities. It also possesses immunomodulatory effects which are of benefit in type II immune complex diseases (see Chapter 57). Danazol probably directly inhibits the synthesis of sex steroids. However, the most prominent action of danazol is suppression of gonadotropins secretion from pituitary, which causes inhibition of testicular/ovarian function.

Danazol is primarily used in human medicine for the treatment of endometriosis, fibrocystic breast disease, gynaecomastia and idiopathic thrombocytopenic purpurea. In veterinary medicine, it used mainly as adjunctive therapy (with corticosteroids) in the treatment of canine immune-mediated thrombocytopenia and haemolytic anaemia (see also Chapter 57). Danazol has, like most other androgenic agents, been linked with an increased risk of liver tumours.

Mesterolone

Mesterolone is a dihydrotestosterone derivative. It possesses weak androgenic properties and hence is rarely used in hormonal replacement therapies.

2. Selective Androgen Receptor Modulators

Selective androgen receptor modulators (SARMs) or mixed androgen receptor agonist /antagonist are a novel class of androgen receptor ligands. They are intended to have the same kind of effects as other androgenic drugs, but their action is more selective at androgen receptors in different tissues i.e. they could act as a partial agonist at certain sites and as an antagonist at other sites.

Andarine

Andarine is a selective androgen receptor modulator (SARM) designed for treatment of conditions such as muscle wasting, osteoporosis and benign prostatic hypertrophy. In investigational studies, andarine has shown to reduce prostate weight, but without producing any reduction in muscle mass or anti-androgenic side effects. This suggests that it competitively blocks binding of androgens to its receptor targets in the prostate gland, but its partial agonist effects at androgen receptors prevent the side effects associated with the anti-androgenic drugs traditionally used for treatment of benign prostatic hypertrophy. Andarine is currently not available for clinical usage, as it is in the clinical trials.

Ostarine

Ostarine is another selective androgen receptor modulator (SARM) designed for treatment of conditions such as muscle wasting and osteoporosis. Similar to andarine, it is in clinical trials.

B. Anabolic Steroids/Drugs Producing Primarily Anabolic Effects

The anabolic steroids are synthetic analogues of testosterone which have enhanced anabolic effects with reduced androgenic activity. The anabolic : androgenic ratio of testosterone is considered as 1; compounds with ratio >1 are called anabolic

steroids (Table 52.1). The selectivity of these steroids is only relative and this may be lost with high doses or prolonged treatment. Alkylation at 17-carbon position impairs the hepatic biotransformation and prolongs the elimination time. Accordingly many anabolic steroids have an alkyl (e.g. methyl) group at the 17-carbon position.

Anabolic steroids are indicated mainly to promote nitrogen retention in animals with catabolic diseases. They are available commercially as oral and/or parenteral preparations, including oil-based products intended for slow release.

1. Androstane

Androstane are organic compounds possessing steroidal nucleus, from which androgens are derived. These compounds thus possess carbon-19 of androgens and are structurally related to testosterone. Androstane compounds are widely used as anabolic steroids in both human and veterinary practices. A large number of these compounds are abused by athletes and bodybuilders for performance enhancement.

Stanozolol

Stanozolol is a synthetic anabolic steroid derived from testosterone. It is alkylated at C-17, which decreases its metabolism and enhances duration of action. Stanozolol has been approved for use in both animal and human patients for a number of conditions. In veterinary medicine it is used mainly for growth promotion, muscle build-up and reversal of tissue depletion in animals. Stanozolol is one of the anabolic steroids commonly used as a performance enhancing drug and is banned from use in sports competition. Stanozolol products are, therefore, controlled substances in several countries.

Mechanism of action

The mechanism of action of stanozolol and other anabolic steroids are similar to those of testosterone and is mediated through the same receptors as the androgenic effects. Stanozolol is predominantly anabolic but retains weak androgenic activity.

Pharmacological effects

Stanozolol and other anabolic steroids stimulate protein synthesis and enhance muscle development resulting in weight gain. Stanozolol initiates and maintains a positive nitrogen balance by increasing nitrogen intake (by stimulating appetite) and decreasing its urinary excretion. The retained nitrogen is incorporated into amino acids and proteins leading to growth, regeneration and hypertrophy of body tissues. This anabolic effect is significant in debilitating and wasting conditions, but is not useful in normal animals or in the acute phase of disease. In all cases, improved nitrogen balance depends on adequate intake of proteins and calories and on treatment of the underlying cause.

Anabolic steroids cause retention of sodium, calcium, potassium, chloride, sulphate and phosphate. They also stimulate appetite, retain intracellular waters, increase muscle mass and enhance production of erythrocytes. The erythropoietic effect of anabolic steroids, however, requires adequate erythropoietin levels and sufficient cells in the bone marrow (see for detail Chapter 36). As part of their anabolic effects, anabolic steroids also antagonise glucocorticoid induced protein catabolism by competitively inhibiting glucocorticoid binding to glucocorticoid receptors.

As stanozolol possesses weak androgenic effect and is structurally related to testosterone, it may suppress release of gonadotropins from anterior pituitary. Large doses may impede spermatogenesis by negative feedback inhibition of FSH and endogenous testosterone release may be suppressed through inhibition of LH.

Pharmacokinetics

Detailed pharmacokinetic information on stanozolol in domestic animals is lacking. Stanozolol is well absorbed after intramuscular administration; it may also be administered orally. The C17 α-alkylation of the drugs decreases the hepatic first pass-metabolism, allowing a high oral bioavailability. The effects of stanozolol persist for at least 1 week in small animals and horses.

Side effects/Adverse effects

Side/Adverse effects of stanozolol in recommended dosage is only mild. Potential adverse effects could be seen when it is used with extensively high doses for a prolonged period of time. These effects include electrolyte and water retention, hepatotoxicity, behaviour changes, and infertility or oligospermia. Excessive use of anabolic steroids in human athletes has resulted in testicular atrophy, sterility and gynaecomastia in men, and inhibition of ovulation, hirsutism, alopecia and acne in women. Anabolic steroids in humans have also been associated with increased risk of coronary heart disease. These effects, however, have not been reported in animals probably due to less frequent use of anabolic steroids.

Contraindications and precautions

Contraindications of stanozolol are similar to those of testosterone. It should not be used in pregnant animals, in hepatic and renal dysfunctions, hypercalcaemia, cardiac insufficiency, prostatic carcinoma, etc. Stanozolol should not be used in pre-pubertal animals because it may close growth plates pre-maturely. Stanozolol and other anabolic steroids should not be used inadvertently. Use of anabolic steroids in food animals is not recommended. Their misuse to gain a competitive advantage in performance animals should be avoided.

Drug interactions

Drug interactions of stanozolol are similar to those of other androgenic steroids mentioned earlier.

Clinical uses

In veterinary medicine, stanozolol is approved for use in small animals and horses. It is used as an adjunct to the treatment of chronic renal failure, in debilitating diseases and convalescence, and to promote tissue repair. It is also used in the treatment of chronic anaemia, particularly those associated with renal disease. Occasionally, stanozolol is used to improve appetite and promote weight gain in animals. Treatment with anabolic steroids may be continued for several weeks, depending on the response and condition of the animal.

Dose

For debilitating conditions (as an anabolic)

Dogs	:	1 – 4 mg (total), PO, 2 times daily.
	:	25 – 50 mg (total), deep IM, once per week.
Cats	:	1 – 2 mg (total), PO, 2 times daily.
	:	25 mg (total), deep IM, once per week.
Horses	:	0.55 mg/kg, deep IM, once per week.

For chronic anaemia

Dogs & cats : 1 – 4 mg (total), PO, once daily.

Boldenone

Boldenone (Dehydrotestosterone) undecylenate is a synthetic testosterone derivative anabolic steroid that has been developed for veterinary use, mostly for use in horses. The activity of boldenone is mainly anabolic, with a low androgenic potency. It increases nitrogen retention, enhances protein synthesis, stimulates appetite and causes release of erythropoietin in kidneys. Commercially, boldenone undecylenate is available in a sesame oil vehicle. Its effect lasts for about 8 weeks after single IM injection.

The side/adverse effects of boldenone are similar to those of other anabolic steroids. Due to its prolonged action, boldenone undecylenate may produce detrimental effects on the reproduction in both male and female animals. Therefore, it should not be used in either stallion or non-pregnant mares intended for reproduction. It is mainly used as adjunctive therapy for treating debilitated horses, when improvement in weight, hair coat or general physical condition is desired. The undecylenate ester greatly extends the activity of boldenone, so that clinically injections would need to be repeated every three or four weeks. Boldenone is not an ideal steroid for the athletes, because it produces certain metabolites which can be detected in urine months after the drug use.

Dose

Horses : 1:1 mg/kg, IM, every 3 weeks; may be repeated at 3 weeks interval.

Fluoxymesterone

Fluoxymesterone is a 17-alkyl substituted derivative of testosterone. Fluoxymesterone is effective when given orally and has a 1 : 2 androgenic to anabolic ratio. In veterinary medicine, fluoxymesterone is used as an anabolic and haematinic agent. Like other anabolic steroids, fluoxymesterone is indicated for the treatment of chronic, non-regenerative anaemias which fail to respond to erythropoietin. Because fluoxymesterone possesses significant androgenic effect, it is also used as an androgenic steroid. Hepatotoxicity is the most serious adverse effect

Dose

For anabolic effects/chronic anaemia

Dogs : 0.2 – 1 mg/kg, PO, once daily.

Oxymetholone

Oxymetholone is a synthetic anabolic steroid used for treatment of osteoporosis and anaemia in human beings. It is also recommended for stimulating the muscle growth in undernourished or underdeveloped patients. It is occasionally used in small animals for promoting gains in body mass, mostly by greatly improving protein synthesis.

Dose

Dogs & cats : 1 mg/kg, PO, once daily.

Other drugs

A large number of androstane anabolic steroids are available commercially and are abused as performance enhancer by sportspersons. These include adrenosterone, androisoxazole, bolasterone, bolazine, boldione, calusterone, 4-chlorodehydromethyltestosterone, clostebol, desoxymethyltestosterone, drostanolone (dromostanolone), enestebol, epitiostanol, formebolone, furazabol, 4-hydroxytestosterone, hydroxystenozole, mebolazine, mesabolone, mestanolone, mesterolone, metenolone (methenolone), methandrostenolone (metandienone, methandienone), methandriol, methasterone, oxandrolone, oxymesterone, prostanozol, quinbolone, roxibolone, silandrone, stenbolone, 1-testosterone and tiomesterone, The anabolic steroidal drugs have no/limited importance in veterinary practice.

2. Estrenes /19-Nortestosterone Derivatives

Estrenes anabolic steroids are similar in structure to the testosterone, but lack carbon at position 19. These compounds are similar in action to androstane anabolic steroids, but may possess some progestational activity due to their resemblance to 19-nortestosterone progestogens.

Nandrolone

Nandrolone (19-Nortestosterone) is a non-alkylated anabolic steroid that is available commercially as nandrolone decanoate (mainly) and nandrolone phenylpropionate. It is used mainly to stimulate erythropoiesis in certain anaemias in both human and veterinary medicines.

Pharmacological effects

Nandrolone, like other anabolic steroids, promote body tissue building process and can reverse catabolism in the presence of adequate protein and calories. Among the anabolic steroids, nandrolone decanoate has the greatest haematopoietic effect. It is believed that nandrolone may enhance red cell count by directly stimulating red cell precursors in the bone marrow and increasing erythropoietin production in the kidney (see for detail Chapter 36). Nandrolone also stimulates appetite and has weak androgenic effects.

Pharmacokinetics

Detailed pharmacokinetics on nandrolone in domestic animals is lacking. Nandrolone esters in oily solutions are administered IM and are generally repeated at 1 week intervals.

Side effects/Adverse effects

Side/Adverse effects and contraindications of nandrolone are similar to those discussed earlier for other anabolic steroids. Virilism, retention of electrolyte and water, hepatopathy, behavioural (androgenic) changes and reproductive abnormalities in both male and female animals are possible with high and/or prolonged use. Nandrolone should not be used in pregnancy because of possible foetal masculinisation. Non-alkylated steroids in general are less hepatotoxic than the alkylated products.

Clinical uses

In veterinary medicine, nandrolone is used mainly to stimulate erythropoiesis in some cases of anaemia (e.g. aplastic anaemia secondary to renal failure). It is used also in the supportive management of chronic renal failure. Nandrolone and other anabolic steroids have been used in conjunction with anticancer chemotherapeutic drugs in order to protect bone marrow production of RBCs. Use of anabolic steroids for cancer has not been established in dogs and cats. Occasionally, nandrolone is used as an appetite stimulant in dogs and as a growth promoter in farm animals (see Chapter 75).

Dose

Dogs & cats : 1 – 5 mg/kg, IM, once daily.

Cats : 10 – 20 mg (total), IM, once daily.

Trenbolone

Trenbolone is a potent anabolic steroid in the 19-nortestosterone family. It is mainly administered as ester

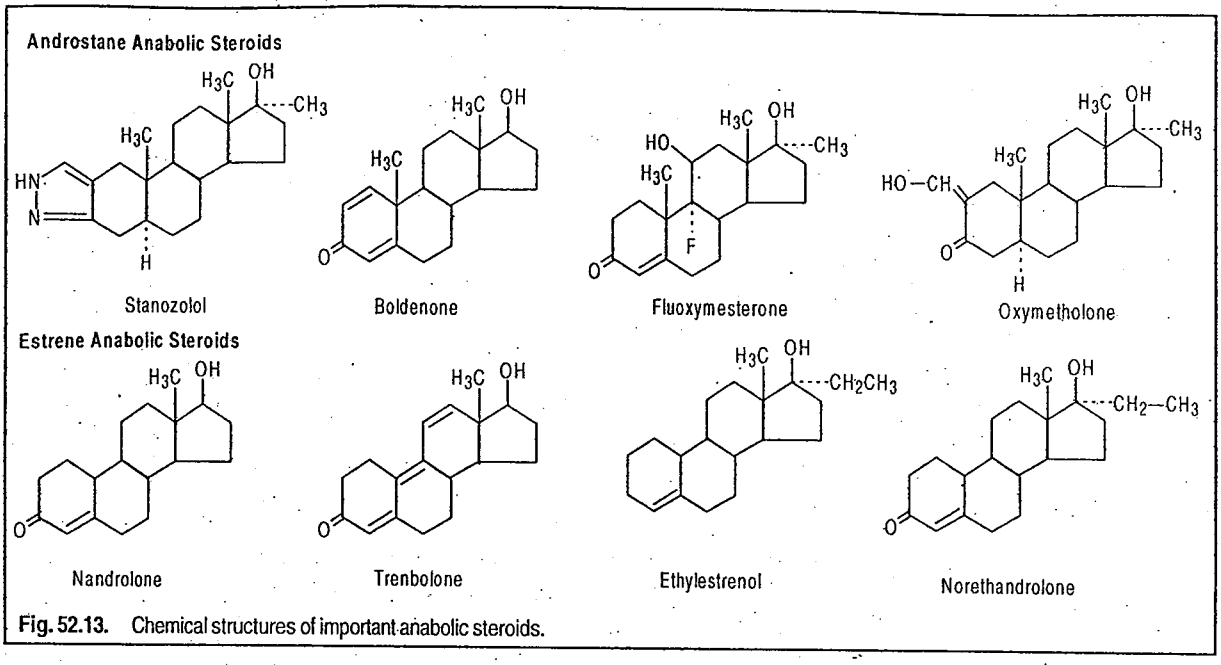


Fig. 52.13. Chemical structures of important anabolic steroids.

derivatives such as trenbolone-acetate, -enanthate or -cyclohexyl-methylcarbonate. After administration, plasma lipases cleave the ester group, causing of release of free trenbolone in the blood stream. The free trenbolone then binds to the androgen receptor and produces anabolic effect of increasing nitrogen uptake by muscles, leading to an increase in the rate of protein synthesis. It also has the secondary effects of stimulating appetite, reducing amount of fat and decreasing rate of catabolism.

Trenbolone is metabolised in liver into 17epi-trenbolone. Both trenbolone and its metabolite are excreted mainly as glucuronide or sulphate conjugates. Unlike many other anabolic steroids, it is not metabolised by aromatase or 5α-reductase into oestrogenic compounds such as estradiol, or into dihydrotestosterone. This greatly reduces the side/adverse effect of water retention normally associated with highly androgenic steroidal compounds. Other side/adverse effects may be similar to other anabolic steroids. In veterinary practice, trenbolone is largely recommended to increase muscle growth used (as growth promoter) and appetite in farm animals.

Ethylestrenol

Ethylestrenol is a nandrolone derivative that lacks the 3-keto functional group. It is a potent anabolic steroid with some progesterone-like activity, and little androgenic activity. It aromatizes only slightly, so oestrogen related side effects are rarely a concern. Water retention is also not commonly seen, although some androgenic effects may be observed. Ethylestrenol is rarely used in animals to promote anabolic effects.

Dose

Dogs & cats : 0.05 mg/kg, PO, daily in divided doses.

Other drugs

A number of other 19-nortestosterone steroids are available commercially and are used for anabolic and/or haematinic purposes. These include preparations of bolenol, ethyldienolone, mibolerone, methylestrenolone, metribolone, norbolethone, norclostebol, norethandrolone, oxabolone cipionate, propetandrol, tetrahydrogestrinone and trestolone. These drugs are mainly used in human beings, but some are also available for use in animals.

Table 52. 1: Comparative anabolic : androgenic ratio of important androgens.

Steroid	Anabolic : Androgenic Ratio
Testosterone	1 : 1
Testosterone esters	1 : 1
Stanozolol	6 : 1
Fluoxymesterone	2 : 1
Methandrostenolone	3 : 1
Oxandrolone	13 : 1
Oxymetholone	3 : 1
Nandrolone decanoate	4 : 1
Ethylestrenol	8 : 1

3. Prohormones of anabolic steroids

Prohormones of anabolic steroids are a relatively different group of drugs used to enhance the hormonal levels in body. These precursors are designed to be converted to full, active steroid hormones via an enzymatic process that occurs during metabolism. These agents are mainly used in sports medicine by athletes to increase muscle size, strength and endurance and to reduce body fat levels. Prohormones have the same side/adverse effects as other anabolic steroids.

4-Androstenedione

4-Androstenedione (Androstenedione) is the same 19-carbon steroid hormone that is produced in the adrenal glands and gonads as an intermediate step in the biochemical pathway leading to biosynthesis of the androgen testosterone and the estrogens estrone and estradiol. After ingestion, androstenedione has been shown to increase serum levels of both testosterone and oestrogens due to its metabolic conversion in body. Adverse effects related to increased levels of oestrogen and testosterone such as gynaecomastia and enlarged prostrate in men are important concern. Androstenedione is available commercially as a dietary supplement, often called *andro* (or *andros*) for short. Currently the sale of the drug has been banned by several countries due to significant health risks commonly associated with steroids.

4-Androstenediol

4-Androstenediol (4-AD) is another hormonal precursor that is converted in body to testosterone. Its rate of conversation to testosterone is about 15.76%, almost three times that of androstenedione, due to utilization of a different enzymatic pathway. There is also no direct conversion to oestrogen, though some secondary aromatization has been reported through metabolism. It appears to be less androgenic than 4-androstenedione, since it does not metabolize into the potent androgen dihydrotestosterone (DHT).

Other drugs

Some other anabolic steroid precursor occasionally used in human medicine include 19-norandrostenedione, 1,4-androstadienedione and 1-testosterone. The 19-norandrostenedione is a precursor of the anabolic steroid nandrolone (nortestosterone) and 1,4-androstadienedione is the precursor of boldenone. The 1-testosterone is a structural analogue of testosterone with a 1,2-double bond instead of a 4,5-double bond in testosterone.

II. Drugs used in Excess of Androgens/Anti-Androgens

Antiandrogens are drugs which block the synthesis or action of androgens. These drugs are useful in the management of hyperplasia and carcinoma of prostrate in males and virilising syndrome in females. In human medicine, antiandrogens may also be considered useful in acne, precocious puberty in boys, and in inhibition of sex drive in man who are sex offenders.

1. Inhibitors of Androgen Synthesis

a. 5α-Reductase Inhibitors

5α-Reductase inhibitors (or 5-alpha-reductase inhibitors) are a group of drugs which block 5α-reductase enzyme and decrease conversion of testosterone to more active form dihydrotestosterone. These drugs are mainly used in treatment of benign prostatic hyperplasia and androgenic alopecia.

Finasteride

Finasteride is a relatively new 4-azasteroid synthetic drug that inhibits 5α-reductase enzyme and decreases intracellular concentration of dihydrotestosterone, the active metabolite of testosterone. Finasteride is mainly used in human medicine in the treatment of prostatic hyperplasia and has also been tried in canine medicine.

Mechanism of action

Finasteride selectively and competitively blocks the 5α-reductase (preferentially type 2, but also type 1) enzyme. This enzyme is responsible for metabolising testosterone to more active dihydrotestosterone (DHT) in prostrate, hair follicles, liver and some other tissues. Since the conversion of testosterone to dihydrotestosterone is blocked in prostrate and other tissues, the androgenic action of testosterone also declines significantly in such tissues. The DHT is a potent hormone and is the primary hormone responsible for development of the prostrate.

Pharmacological effects

Finasteride blocks the synthesis of dihydrotestosterone and causes significant reduction in prostrate size and improves urine flow in patients with prostatic hyperplasia. Finasteride administration usually maintains the sperm production because it mainly decreases intraprostatic dihydrotestosterone concentration (at spermatogenic cells in testes, testosterone itself is the active androgen). It causes profound reduction in the concentration of DHT in plasma, but does not alter plasma testosterone or LH levels

Pharmacokinetics

Pharmacokinetics data on finasteride in domestic animals are lacking. In humans, finasteride is absorbed after oral administration and about 90% is bound to plasma proteins. It is well distributed in body and is found in seminal fluid. It is metabolised in the liver and its half-life is about 6 hours. In humans, a single daily dose suppresses dihydrotestosterone for about 24 hours.

Side effects/Adverse effects

In humans, finasteride is reported to produce decreased libido, decreased ejaculate volume and impotence. Overall adverse effects are limited, mild and transient. No information on adverse effects in domestic animals has been available. Finasteride administration in pregnant females has been associated with teratogenic effects. Occasionally, it results in allergic reactions like skin rashes.

Contradictions and precautions

Finasteride should not be used in pregnant animals and in males meant for breeding purposes. It should be used with caution in patients with significant hepatic impairment. Withdrawal of the drug generally results in regrowth of prostrate.

Clinical uses

Finasteride is used in humans to treat benign prostatic hypertrophy and has proved successful in dogs also. However, its use is limited in veterinary practice mainly due to its high cost and requirement of long duration of therapy.

Dose

Dogs : 5 mg/kg, PO, once daily.

Dutasteride

Dutasteride is a 5-alpha-reductase inhibitor that blocks the conversion of testosterone into dihydrotestosterone (DHT). It is similar in action to finasteride, with only difference that dutasteride inhibits both isoforms of 5-alpha reductase, Type I and Type II, whereas finasteride inhibits only Type II. It has been approved for the treatment of benign prostatic hyperplasia (BPH).

b. CYP17A1 Inhibitors

CYP17A1 Inhibitors are a novel class of anti-androgenic drug that blocks CYP17A1, a cytochrome P-450 enzyme involved in the formation of endogenous 19-carbon steroids dehydroepiandrosterone and androstenedione, which may ultimately be metabolized into testosterone. Abiraterone acetate, a CYP17A1 inhibitor, is currently in advanced clinical trials for treatment of prostate cancer resistant to commonly used anti-androgenic drugs.

C. Gonadotropin-Releasing Hormone and Synthetic Analogues

Gonadorelin

Gonadorelin is a synthetic human gonadotropin-releasing hormone (GnRH). Continuous administration of gonadorelin or its synthetic analogues (e.g. leuproline) are reported to produce inhibitory effect on LH, which in turn suppresses gonadal steroid synthesis. The level of testosterone declines and produces a reversible pharmacological castration. These drugs have been tried by producing androgen deprivation in men with prostate cancer who cannot tolerate diethylstilbestrol. In veterinary

medicine, gonadorelin and its synthetic analogues are not used for anti-androgenic purposes. The pharmacology of gonadotropin-releasing hormone has been described in Chapter 49.

D. Miscellaneous Drugs

Ketoconazole

Antifungal agents like ketoconazole inhibit synthesis of androgens by blocking cytochrome P-450 enzymes involved in steroid hormone synthesis. These are occasionally used in human beings to induce androgen deprivation in some patients with prostate cancer. The pharmacology of ketoconazole and other antifungal drugs has been described in Chapter 66.

Spironolactone

Spironolactone, an aldosterone antagonist, inhibits androgen synthesis and also acts as a weak androgen receptor antagonist. It is used rarely in women to control excessive hair growth and hirsutism. It is also used for treating acne in women and can be used as a topical medication for treatment of male baldness.

2. Androgen Receptor Antagonists

Cyproterone acetate

Cyproterone acetate is a synthetic derivative of 17-hydroxyprogesterone that acts as an androgen receptor antagonist with weak progestational and glucocorticoid activity. Its main indications are prostate cancer, benign prostatic hyperplasia, priapism, hypersexuality and other conditions in which androgen action maintains the disease process.

Mechanism of action

The mechanism of action of cyproterone is complex. It is primarily an antagonist at androgen receptor that blocks the action of endogenous androgens (anti-androgenic activity). Cyproterone is chemically related to progesterone and has mild progesterone receptor stimulant effect (progestational activity). On glucocorticoid receptors, its action is both agonistic and antagonistic; the net activity is minor glucocorticoid effect. The progestational and glucocorticoid effects reduce production of gonadotropins, which in turn results in lower testosterone levels (anti-androgenic effect). Cyproterone acetate is also an inhibitor of the steroidogenic enzyme 21-hydroxylase and to a lesser extent 3-beta-hydroxysteroid dehydrogenase, both of which are needed to synthesize hydrocortisone (anti-glucocorticoid activity) and also aldosterone (anti-mineralocorticoid activity). The blockade of adrenal 21-hydroxylase results in the accumulation of androgen precursors which may be converted to testosterone (androgenic effect); this reduces the androgen receptor blockade action. The overall action of cyproterone acetate is considered to be moderate anti-androgenic with weak progestational and glucocorticoid activity.

Pharmacological effects

The pharmacological effect is primarily produced by the cyproterone acetate that has approximately three times the anti-androgenic activity of cyproterone. It reduces action of endogenous androgens at different sites including the prostate gland. In addition, it suppresses LH and FSH secretion from the anterior pituitary. Larger doses produce effects similar to castration.

Pharmacokinetics

Cyproterone acetate is a lipophilic agent and its pharmacokinetics is complex due to its storage in adipose tissues. In human beings, its mean elimination half-life is about 40 hours, but is affected by the amount of drug stored in the fat. Pharmacokinetics data on cyproterone acetate in domestic animals are lacking.

Side/Adverse effects

Important side/adverse reported in human patients include hepatotoxicity, especially in high doses. It may suppress adrenal function with low levels of hydrocorticosterone, which may impair carbohydrate metabolism. Its anti-androgenic effect may produce gynaecomastia, galactorrhoea and erectile dysfunction in men. Long-term treatment suppresses production of oestrogen due to its antigonadotropic effect, which may result in osteoporosis.

Clinical uses

Cyproterone acetate has been used in humans in the treatment of precocious puberty and prostrate hyperplasia and carcinoma. It is also used to inhibit libido in men with severe deviations of sexual behaviour. Due to its anti-androgen effect, it can also be used to treat hirsutism, and is a common component in hormone therapy for male-to-female transsexual people.

Flutamide

Flutamide is a non-steroidal anti-androgenic compound. It is biotransformed *in vivo* to an active metabolite 2-hydroxyflutamide, which competitively blocks binding of dihydrotestosterone to the androgen receptor in the accessory sex organs (e.g. prostate). It also blocks the inhibitory action of androgens on pituitary leading to increased plasma levels of LH and testosterone. The increased testosterone levels partially overcome the antiandrogenic action of flutamide. In human medicine, flutamide is used in advanced prostatic carcinoma in conjunction with a GnRH agonist or oestrogen to suppress LH and testosterone secretion. Important side/adverse effects associated with flutamide are gastrointestinal side effects. Prolong treatment may cause gynaecomastia in men.

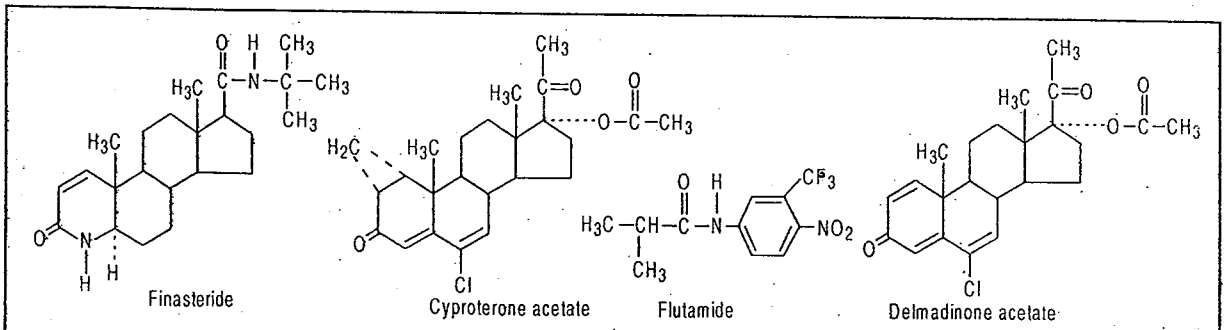


Fig. 52.14. Chemical structures of selected antiandrogenic compounds.

Other drugs

Bicalutamide and nilutamide are two other non-steroidal androgen receptor antagonists. Both are used as anti-androgen drugs in the treatment of prostate cancer in human medicine.

3. Female Sex Hormones

Estrogens and some progestogens have antiandrogenic action, but are used infrequently in clinical practice. On prolonged use, they lead to castration like actions.

Delmadinone

Delmadinone acetate is a synthetic progestogen, which possesses significant antiandrogenic activity. It competes with androgens for receptor sites and produces its pharmacological effects. Delmadinone also acts upon the limbic system to reduce behavioural effects and may markedly reduce aggression in some dogs (see also Chapter 23). Delmadinone may produce transient reduction in fertility and libido in treated animals, in addition to other adverse effects noted with progestogens. Delmadinone acetate is used mainly in veterinary practice in the treatment of prostatic hypertrophy, prostatic carcinoma, perianal tumours and hyper sexuality in dogs and cats. Delmadinone should be used with care in animals intended for future breeding use or those receiving other steroid concurrently.

Dose

Dogs & cats : 1 – 2 mg/kg, SC or IM. If no improvement is seen, dose may be repeated after 8 days. In animals showing improvement, drug is repeated every 3-4 week.

Dienogest

Dienogest is an orally active synthetic progestogen with significant anti-androgenic activity. It is largely used in the treatment of endometriosis, but can improve androgenic symptoms as well.

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CHAPTER 53 PANCREATIC HORMONES

Insulin
- Synthesis, release, transport and fate
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- Mechanism of action
- Physiological effects
- Disorders of insulin
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    - Biguanides
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    - Thiazolidinediones
      - Mechanism of action
    - Miscellaneous drugs
      - Alpha-glucosidase inhibitors
      - Amylin analogues
      - Sodium-dependent glucose co-transporters inhibitors
      - Other drugs

Glucagon
- Synthesis, release and fate
- Regulation of secretion
- Mechanism of action
- Physiological effects
- Disorders of glucagon
  - Deficiency of glucagon
  - Excess of glucagon
- Treatment of glucagon disorders
  - Drugs used in deficiency of glucagon
  - Drugs used in excess of glucagon

Pancreas is a glandular organ that has both exocrine and endocrine roles. The exocrine portion of pancreas secretes pancreatic juices consisting of digestive enzymes, electrolyte and water and is mainly concerned with GI functions. The endocrine portion of pancreas, namely the islet of Langerhans, contains cells which secrete peptide hormones responsible for maintaining stable concentrations of glucose and other nutrients in the body.

The endocrine pancreas is organised as discrete islet (islets of Langerhans) that contains four cell types, alpha (α), beta (β), delta (δ) and F or PP cells, each of which produces a different hormone. Most numerous (~60%) of islets are the β cells which secrete insulin and the α-cells (~25%) those produce glucagons. The δ or D-cells secrete somatostatin and F or PP cells produce a hormone of uncertain function called pancreatic polypeptide. Although these hormones have different functions, they are involved in the control of metabolism, more particularly in glucose homeostasis.

INSULIN

Insulin is a hormone synthesised by the β cells in the islet of Langerhans and derives its name from a Latin word '*insula*', which means island. It consists of two peptide chains A and B, which are linked by two disulphide bridges. The A chain contains 21 amino acids and the B chain contains 30 amino acids. Although the monomer form of insulin is thought to be the active form, it also exists in dimer and hexamer forms, the latter being complexed with two zinc molecules. Among various domestic animals, feline insulin is most similar to bovine insulin, and canine and porcine insulin are identical and are similar to human insulin. Overall, the biological activities of insulin are not highly species specific mainly due to minor interspecies differences in the structure of insulin. Insulin is involved mainly in carbohydrate metabolism by causing cells in the liver, muscle and adipose tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle.

Synthesis, Release, Transport and Fate

Synthesis and release: The synthesis of insulin is similar to that of other peptide hormones. It begins with the formation of a linear polypeptide pre-proinsulin (Mol. wt. 11,500) within the rough endoplasmic reticulum in response to feeding and increase in plasma concentration of glucose reaching the islets. The initial pre-proinsulin is then cleaved to form a proinsulin with a molecular

weight of about 9000. The proinsulin is transferred to Golgi apparatus, where it is further cleaved to form insulin (Mol. wt. 6000) before being packaged in the secretory granules. The release of insulin involves biphasic kinetics – an initial phase reflecting release of stored hormone and a slower delayed phase reflecting both release of stored hormone and new synthesis. The release process involves mainly exocytosis in which membranes of secretory granules fuse with the plasma membrane of B cells and insulin contained in cells is freed. The insulin molecules then diffuse into capillaries and enter the portal circulation.

Transport and metabolism: After release, insulin circulates almost entirely in an unbound form. Some portion of insulin combines with the specific insulin receptors in target cells and remainder is degraded by the enzyme insulinase mainly in liver, to a lesser extent in kidneys and muscle, and slightly in most other tissues. Metabolism of insulin involves mainly reduction of the disulphide bonds which link the A and B chains followed by the protease activity, which reduces them to peptides and amino acids. Insulin has a plasma half-life of about 6-10 minutes, so is mainly cleared from the circulation within 10 to 15 minutes.

Regulation of Synthesis and Release

Synthesis and secretion of insulin is mainly regulated by the blood glucose concentration. The β cell responds to both the actual glucose concentration and also the rate of change of glucose concentration. Plasma insulin concentrations increase almost 10 fold within 3 to 5 minutes after the acute elevation of blood glucose. Glucose enters the β-cells through the glucose transporter GLUT2. Glucose then goes into glycolysis and the respiratory cycle, where multiple high-energy ATP molecules are produced by oxidation. The metabolism of glucose and increase in the concentration of ATP leads to closure of the K^+-channels in the cell membrane causing depolarisation. This in turn opens voltage dependent Ca^{++} channels leading to Ca^{++} influx. Under the influence of increased Ca^{++}, the secretory granules fuse and release insulin by the process of exocytosis.

Stimuli to insulin release other than glucose include glucagon, amino acids (particularly arginine and leucine), fatty acids and various hormones from GI tract. The GI hormones such as gastrin, secretin, cholecystokinin, gastric inhibitory polypeptide (GIP), vasoactive intestinal polypeptide (VIP) and enteroglucagon released by eating stimulate insulin secretion, whereas somatostatin and amylin inhibit insulin secretion. Glucagon-like peptide-1 (GLP-1), a gastro-intestinal hormone (often called incretin) secreted by endocrine cells located in the epithelium of small intestine, also play a major role in release of insulin from the beta cells of the islets of Langerhans after eating, even before blood glucose levels become elevated. Release of insulin is stimulated by parasympathetic stimulation, while adrenergic stimulation or epinephrine release inhibits insulin secretion. Catecholamines tend to decrease insulin secretion thorough an interaction with the alpha-adrenoceptors on the β cells. Hypercalcaemia promote insulin release, while hypocalcaemia decrease insulin release. Butyrate and, to a lesser degree, propionate are potent stimulators of insulin release in ruminants.

Insulin, glucagon and somatostatin modulate the release of one another through a paracrine relationship. Glucagon and related peptides stimulate insulin secretion, while insulin inhibits glucagon release. Somatostatin inhibits the release of both insulin and glucagon, while glucagon stimulates somatostatin release. A number of hormones such as growth hormone, corticosteroids and thyroxine modify insulin release in response to glucose. Prostaglandin E (PGE) has shown to inhibit insulin release.

Mechanism of Action

Insulin acts on specific receptors located on cell membrane of the target cells. Practically all cells of body contain insulin receptors, but their density is particularly very rich in liver and fat cells. The insulin receptors (Mol. wt. 300,000) belong to tyrosine kinase receptors superfamily and consists of four subunits – 2 α- and 2 β-subunits held together by disulphide linkages. The alpha subunits those lie entirely outside the cell membrane carry insulin-binding site, while beta subunits which protrude into the cytoplasm have tyrosine protein kinase activity. Binding of insulin to the alpha-subunits activates tyrosine kinase activity of the beta-subunits i.e. tyrosine residues of the β-subunits get autophosphorylated and become an activated enzyme (see for detail Chapter 6). It has been suggested that binding of insulin to α-subunits actually induces aggregation and internalisation of the receptor along with the bound insulin molecules, which in turn activate tyrosine kinase activity of the β subunits. The autophosphorylation and activation of enzyme causes

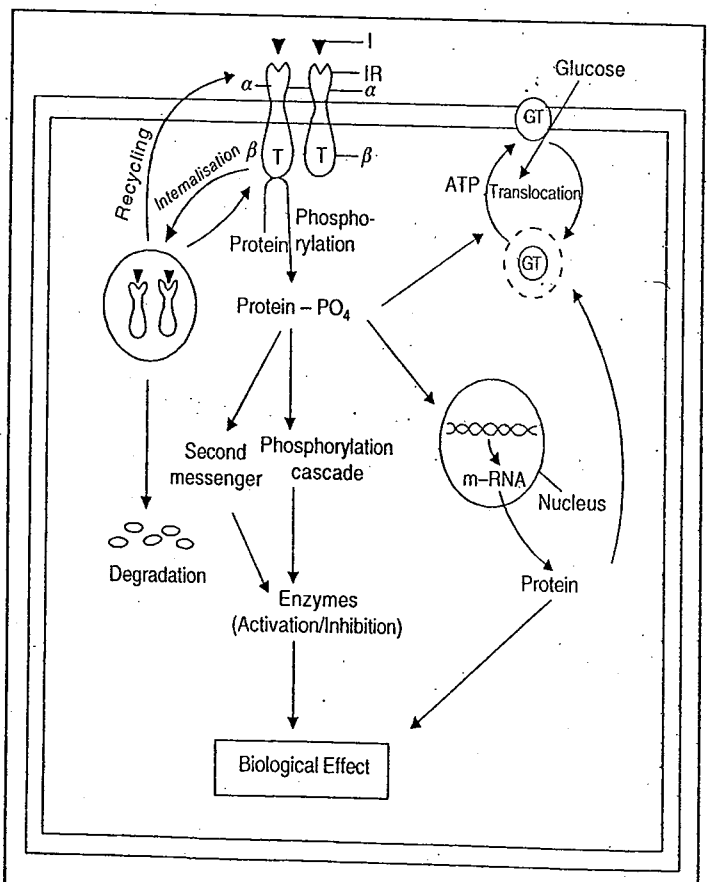


Fig. 53.1. Schematic representation of mechanism(s) of action of insulin (See text for detail).

Abbreviations : I = insulin; IR = insulin receptor; α = alpha subunit; β = beta subunit; GT = glucose transporter; ATP = adenosine triphosphate; m-RNA = messenger ribonucleic acid; T = tyrosine residues.

phosphorylation of multiple other intracellular enzymes leading to either activation or inhibition of enzymes involved in the metabolic actions of insulin. Certain second messengers like phosphatidyl inositol glycan (PIG) and diacylglycerol (DAG), which are generated through activation of a specific phospholipase C, are also believed to mediate action of insulin on metabolic enzymes.

The end effects of insulin receptor stimulation may include enhanced facilitated transport of glucose across the cell membrane within seconds or minutes. Insulin enhances entry of glucose mainly by ATP dependent translocation of intracellular glucose transporters (GLUT) to the plasma membrane as well as by increasing their activity. Over a period of time it also appears to be needed for the expression of genes directing synthesis of the glucose transporters. The internalised receptor-insulin complex is either degraded or returned back to the surface from where the insulin is released extracellularly (Figure 53.1).

Physiological Effects

Insulin acts at a number of sites within the metabolic pathways of carbohydrates, fats and proteins and control intermediary metabolism. It affects every tissue in the body, but effect is profound on liver, muscle and fat cells. The overall effects of insulin are to favour storage of fuel.

Carbohydrate metabolism: Insulin has profound effects on carbohydrate metabolism. Its main function is to facilitate the transport of glucose across the plasma membranes of cells of most tissues including liver, muscle and fat. The exceptions are red and white blood cells and some cells of liver, brain, adrenal and intestine, which require continuous supply of glucose and, therefore, do not depend on insulin. Insulin decreases blood glucose concentration and inhibits the release of glucose from liver. In the liver, insulin decreases glycogenolysis and gluconeogenesis and increases glycogen synthesis and glycolysis (glucose utilisation). Insulin decreases gluconeogenesis by promoting protein synthesis in the peripheral tissues, thereby decreasing the amount of amino acids available for gluconeogenesis, and also by decreasing the activities of hepatic enzymes which are involved in the conversion of amino acids to glucose. In skeletal muscle and adipose tissues, insulin promotes glucose transport and increases glycogen synthesis and glycolysis. Glycogen synthesis in the liver, adipose tissue and skeletal muscle mainly occurs due to induction of glycogen synthetase activity with a concomitant decrease in glycogen phosphorylase activity that decreases glycogenolysis.

Lipid metabolism: In adipose tissues, insulin increases fatty acid synthesis and triglyceride formation and decreases lipolysis. Fatty acid synthesis is facilitated by insulin mainly by enhanced levels of pyruvate (pyruvate is a precursor of acetyl coenzyme A, which is utilised for synthesis of fatty acids). Insulin also causes lipogenesis in liver, which may be even more than that in adipose tissue. Inhibition of lipolysis in adipose tissues mainly occurs due to dephosphorylation and inactivation of lipases by insulin. Insulin also decreases the lipolytic action of epinephrine and growth hormone (and possibly glucagon) by opposing their action.

Protein metabolism: Insulin stimulates uptake of amino acid into muscle and possibly other cells (not liver) and increases protein synthesis. It also decreases protein catabolism and oxidation of amino acids, particularly in the liver. It also increases DNA replication via control of amino acid uptake.

Table 53.1: Effects of insulin on carbohydrate, lipid, and protein metabolism.

Type of Metabolism	Liver	Adipose	Muscle
Carbohydrate metabolism			
Glucose uptake	–	↑	↑
Glycogenolysis	↓	↓	↓
Gluconeogenesis	↓	–	–
Glycogenesis	↑	↑	↑
Glycolysis	↑	–	↑
Lipid metabolism			
Lipogenesis	↑	↑	–
Lipolysis	↓	↓	–
Protein Metabolism			
Amino acids uptake	–	–	↑
Protein synthesis	–	–	↑
Protein breakdown	↓	–	–

Symbols: ↑ = stimulation/increase; ↓ = inhibition/decrease; – = no significant effect.

Other metabolic effects: Insulin increases uptake of potassium ions into cells through activation of the Na^+-K^+-ATPase pump. It also increases transport of Ca^{++}, nucleosides and inorganic phosphate into cells. Insulin causes increase in the secretion of hydrochloric acid by parietal cells in stomach.

Long-tem effects: In addition to immediate actions on metabolism, insulin has long-term action by modifying (increasing or decreasing) the synthesis of key enzymes. It also stimulates cell proliferation *in vitro* and is implicated in growth regulation *in vivo*. Growth hormone and insulin function synergistically to promote growth, each performing a specific function that is probably separate from that of the other.

DISORDERS OF INSULIN

Deficiency of Insulin

Deficiency of insulin occurs due to failure of β-cells to produce sufficient insulin. The pathogenic mechanisms responsible for decreased insulin production and secretion are multiple, but mostly they are related to destruction of islets cells, secondary to either severe pancreatitis or selective degeneration. In dogs, chronic pancreatitis and immune destruction of pancreatic islets are the most common causes of insulin deficiency.

Deficiency of insulin leads to a chronic disorder of carbohydrate metabolism called diabetes mellitus, which is a relatively common endocrinopathic disease in humans and small animals. In small animals, diabetes mellitus has been classified as type 1 and type 2. Type 1 diabetes mellitus, called insulin dependent diabetes mellitus (IDDM), results from the body's failure to produce insulin, although response to insulin may be normal. The type 2 diabetes, called non-insulin dependent diabetes mellitus (NIDDM), results from insulin resistance, despite adequate secretion of insulin in the body. Causes of type 1 diabetes in animals may include hereditary factors and pancreatic destruction by pancreatitis, viral infection or autoimmune disease. Type 2 diabetes may reflect a relative insulin deficiency. The basal insulin levels in type 2 diabetes may be lower or higher than the normal levels, but responses to glucose are usually poor. Other forms of diabetes mellitus include congenital diabetes, which is due to

genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids.

Overt diabetes is diagnosed by persistent hyperglycaemia (>200 mg/dl). Mild hyperglycaemia such as that induced by stress may result in glucosuria. Glucosuria that accompanies hyperglycaemia causes an osmotic diuresis and depletion of sodium chloride and potassium. Common clinical signs in dogs include polydipsia, polyuria and polyphagia with weight loss, bilateral cataracts and weakness. Diabetic animals have decreased resistance to bacterial and fungal infections and often develop chronic or recurrent infections such as cystitis, prostatitis, bronchopneumonia and dermatitis. Hepatomegaly due to lipid accumulation is common in diabetic dogs and cats. Increased catabolism of carbohydrate, protein and fat leads to ketogenesis. Initially ketone bodies are used as fuel source in the glucose-deficient tissues, but slowly they accumulate and disturb body's buffering system leading to metabolic acidosis called diabetic ketoacidosis.

Excess of Insulin

Increased insulin production, known as hyperinsulinism, occurs occasionally from an adenoma of the islet of Langerhans. These neoplasms frequently are hormonally active and secrete excessive amounts of insulin and may also produce somatostatin, pancreatic polypeptide, gastrin and vasoactive intestine peptide. The clinical signs are a reflection of hypoglycaemia and may include weakness, fatigue after exercise, generalised muscular twitching, ataxia, mental confusion and changes of temperament. Periodic seizures may occur and episodes of collapse resembling syncope have been reported.

TREATMENT OF INSULIN DISORDERS

Treatment of insulin disorders are largely confined to management and treatment of insulin deficiency resulting in diabetes mellitus. These agents, often called antidiabetic agents, have primary use in the treatment of diabetes mellitus. The aim of the treatment is to achieve best possible control of plasma glucose concentration. These agents are used along with dietary management to maintain the patient's ideal body weight with normal water consumption and urine output avoiding periods of hypoglycaemia. Excess of insulin secretion, often resulting in hypoglycaemia, may be treated with glucagon, a pancreatic hormone, or non-specific drugs like octreotide and diazoxide.

Classification

I. Drugs used in deficiency of insulin
  A. Insulin and analogues / Parenteral antidiabetic drugs
    1. Short/Rapid acting agents
       e.g. regular insulin, semi-lente insulin, insulin lispro, insulin aspart and insulin glulisine.
    2. Intermediate acting agents
       e.g. isophane (NPH) insulin and lente insulin.
    3. Long/Slow acting agents
       e.g. protamine zinc insulin, ultralente insulin, insulin glargine and insulin detemir.
    4. Ultra-long acting insulin
       e.g. insulin degludec.
  B. Insulin secretagogues
    1. Sulphonylureas /Oral antidiabetic drugs
      a. First generation sulphonylureas
         e.g. chlorpropamide, tolbutamide, tolazamide, acetohexamide and carbutamide.
      b. Second generation sulphonylureas
         e.g. glipizide, glibenclamide, gliclazide, gliquidone, glyclopyramide and glimepiride.
    2. Non-sulfonylurea secretagogues
      a. Meglitinides
         e.g. mitiglinide, repaglinide and nateglinide.
      b. Glucagon-like peptide-1 analogues
         e.g. exenatide and liraglutide.
      c. Dipeptidyl peptidase-4 inhibitors
         e.g. sitagliptin, vildagliptin and saxagliptin.
  C. Insulin sensitizers
    1. Biguanides
       e.g. metformin and phenformin.
    2. Thiazolidinediones
       e.g. pioglitazone, rosiglitazone, englitazone, ciglitazone and troglitazone.
  D. Miscellaneous agents
    1. Alpha-glucosidase inhibitors
       e.g. acarbose, miglitol and voglibose.
    2. Amylin analogues
       e.g. pramlintide.
    3. Sodium-dependent glucose co-transporter inhibitors
       e.g. canagliflozin and dapagliflozin.
    4. Others drugs
       e.g. guar gum and glucomannan.
II. Drugs used in excess of insulin
  e.g. glucagon, octreotide and diazoxide.

I. DRUGS USED IN DEFICIENCY OF INSULIN

A. Insulin and Analogues /Parenteral Antidiabetic Drugs

Insulin is a natural hormone produced and secreted by the pancreatic beta-cells. It is available is a variety of preparations, which vary in their ADME (absorption, distribution, metabolism and excretion) characteristics. Commercial insulin is obtained from bovine, porcine and human sources. Human insulin is produced also by recombinant DNA techniques and is used preferentially in human and veterinary practices. Insulin analogues, the bio-genetically altered form of insulin, are recent addition in the list of insulin preparations. The number and types of insulin preparations available in the market is constantly changing and increasing, but still insulin remains the most frequently used antidiabetic drug in both human and veterinary medicines.

Insulin Preparations

A variety of insulin preparations are available in market, which differ in their source and duration of action. The rate of absorption of insulin and its duration of action depend on the physical state (crystalline or amorphous, proportion of zinc, and solution or suspension), and route of injection of the chosen formulation. However, the rate of absorption, peak serum time and duration of action of various insulin preparations may vary between individuals due to variations in blood flow, injection site, temperature and exercise.

Regular insulin (soluble insulin), prepared by precipitating the insulin with zinc chloride, is unmodified and soluble form of

insulin that acts the same whether crystalline or non-crystalline. Addition of protamine, a basic protein obtained from the testes of fish, to insulin forms a protamine-zinc-insulin (PZI) complex with low solubility and slow absorption of insulin. A related product, Neutral Protamine Hagedorn (NPH) insulin, is also a complex of insulin, zinc and protamine, but is prepared by a modified ratio of protamine and insulin. Concerns over the potential antigenicity of protamine (obtained from fish) in PZI and NPH insulins led to the development of Lente insulins. Lente insulins (insulin zinc suspensions) possess high concentrations of zinc in an acetate rather than phosphate buffer, but do not contain protamine. In case of lente-insulins, the absorption of insulin is controlled by the size of zinc-insulin crystals (e.g. semi-lente is composed of small zinc-insulin crystals from which absorption is rapid, whereas ultra lente contains large zinc-insulin crystals those are absorbed more slowly).

1. Short/Rapid Acting Insulin Preparations

Regular insulin/ Soluble insulin

Regular insulin (soluble insulin, neutral insulin, crystalline insulin, unmodified insulin) is a rapid-acting, unmodified form of insulin obtained from the pancreas of pigs and/or cattle at slaughter. It is purified and prepared by precipitating the insulin with zinc chloride, forming zinc insulin crystals. It is the only insulin that is a clear, colourless solution and suitable for IV administration; all others insulin preparations are suspensions. Human recombinant insulin is available also and is useful in dogs and cats.

Pharmacological effects

The mechanism of action and pharmacological effects of regular insulin is similar to those of endogenous insulin. Regular insulin has rapid onset of action and lowers the blood sugar within minutes. Intravenous administration of regular insulin produces immediate action and is useful in emergency conditions, such as ketoacidosis, diabetic coma and surgical complications.

Pharmacokinetics

All preparations of insulin are inactive orally due to rapid degradation of the polypeptide in the GI tract. Regular insulin is usually given subcutaneously, but may also be used intravenously or intramuscularly. Onset of action occurs within 15-30 minutes of SC or IM injection, peaks in 2-4 hours, and lasts for up to 5-8 hours. When injected IV, soluble insulin has a very rapid but very short half-life; the effect disappears within 2 to 4 hours. In cats and dogs, IV infusion at low dosage produces continuous effects, but action tends to cease immediately when the infusion is stopped.

Insulin is distributed only extracellularly. Injected insulin is metabolised primarily in liver and to a lesser extent in kidneys and muscles. Like endogenous insulin, the injected insulin is biotransformed first by separation of A and B chains and then subsequent metabolism to constituent amino acids.

Side effects/Adverse effects

The main undesirable effect of insulin is hypoglycaemia. Symptoms may include weakness, shaking, head tilting, lethargy, ataxia, seizures and coma. Overdosage of insulin can result in substantial morbidity and death. Insulin shock, the worst sequel of hypoglycaemia, occurs due to deficiency of glucose in the CNS and is characterised by convulsions and coma. Rebound hyperglycaemia (Somogyi effect) can follow excessive insulin administration. This results from release of the insulin opposing or counter regulating hormones in response to insulin-induced hypoglycaemia. Allergy to insulin is not common, but may take the form of local or systemic reactions. Regular and other preparations of insulin are likely to be immunogenic in animals to a greater or lesser extent, but resistance to exogenous insulin action is uncommon. Regular injection of insulin may produce loss or proliferation of adipose tissue at the site of injection. It can be minimised by rotating injection sites.

Treatment: Treatment of insulin overdosage involves management of hypoglycaemia. Mild hypoglycaemia may be treated by offering the animal its normal food, whereas moderate signs may require treatment with sugar or syrup ingested or rubbed on the buccal membranes. More serious signs may require IV injection of 50% dextrose solution. Glucagon may be injected IM, if IV administration of glucose is not feasible.

Contraindications and precautions

There are no absolute contraindications for insulin in animals because there are no satisfactory alternatives for insulin in diabetic patients. If animal develops hypersensitivity or should insulin resistance develop, a change in type of species of insulin should be tried. Insulin resistance develops in some animals either due to development of insulin antibodies which may attenuate responses to exogenous insulin or due to enhanced secretion of stress hormones like epinephrine and corticosteroids. All insulin preparations should be stored in a refrigerator at 2-8 °C because they are adversely affected by heat or freezing. Insulin requirements are increased by infections, oestrus, pregnancy and ketoacidosis. Obesity should be avoided because this will increase insulin resistance.

Drug interactions

A number of drugs alter response to insulin. Anabolic steroids, beta-adrenoceptor blockers, monoamine oxidase inhibitors, NSAIDs and tetracyclines potentiate the hypoglycaemic action of insulin. On the other hand, glucocorticoids, catecholamines, and furosemide and thiazide diuretics may decrease the hypoglycaemic activity of insulin. Because insulin can alter serum potassium levels, concomitant cardiac glycoside therapy should be monitored.

Clinical uses

Regular insulin is used mainly for rapidly resolving diabetic ketoacidosis in dogs and cats and is also useful for the treatment of uncomplicated diabetes mellitus. Treatment of diabetic ketoacidosis often requires correction of dehydration, electrolyte and acid/base imbalances and treatment of precipitating factors (e.g. infection). The rapid onset and short duration profile generally precludes the use of regular insulin in the daily treatment of diabetes because of the number of injections required. Regular insulin can be administered in combination with longer acting products (except protamine zinc insulin) for daily use in diabetes mellitus to achieve rapid and longer duration of action. Occasionally, regular insulin is used for the adjunctive treatment of hyperkalaemia.

Dose

For adjunctive therapy of diabetes ketoacidosis.

Dogs & cats : 0.025 – 0.05 unit/kg/hour, IV infusion (1 unit/100 ml of IV fluid)

: 0.2 unit/kg, IM as loading dose, followed by 0.1 unit/kg, IM every hour until blood glucose drops below 250 mg/dl.

Dogs : 0.5 unit/kg, SC, every 3-8 hours.
Cats : 0.25 unit/kg, SC, every 3-8 hours.

For adjunctive treatment of hyperkalaemia

Dogs & cats : 5 units/kg/hour, IV infusion combined with glucose at 2 gram per unit of insulin.

Semi-lente insulin

Semi-lente insulin (Prompt insulin zinc suspension) is a suspension of amorphous crystalline form of insulin with zinc (about 10 times greater than that in regular insulin) in acetate buffer. It is composed of small zinc-insulin crystals (2 μm) which are absorbed rapidly. The kinetics of absorption of semi-lente insulin and regular insulin are similar, but semi-lente insulin has a comparatively longer duration of action. It is administered only SC and is not suitable for IV administration. After SC injection, its onset of action is 1-2 hours, peak effect occurs in 4-8 hours, and duration of action is 12-16 hours. It can be mixed with soluble insulin and is administered twice daily. Semi-lente insulin is rarely used today.

Insulin lispro

Insulin lispro is a fast acting insulin analogue. It was the first insulin analogue prepared by recombinant DNA methods in which the amino acids at position B28 (proline) and B29 (lysine) in the beta chain of human insulin have been inverted. This structural modification results in more rapid absorption, higher peak concentration and a shorter duration of action. Insulin lispro binding to insulin receptor and immunogenicity are unaffected by the structural change, but it results in decreased formation of insulin dimers and hexamers. This action allows larger amounts of active monomeric insulin to be immediately available for postprandial injections.

Lispro insulin is injected 0-15 minutes before a meal. It results in peak serum insulin concentrations in 30-90 minutes and duration of action of only 3-4 hours. These differences in action allow for more flexibility in dosing with lispro insulin than regular insulin, which requires a longer waiting period before starting a meal after injection. It may also improve the glycaemic control in some patients while decreasing the risk of hypoglycaemia with multiple injection therapy. Insulin lispro is a buffered insulin and is commonly used for insulin therapy in human patients who wear external SC insulin infusion pumps. Insulin lispro is not recommended for IV administration. It may be given in the same syringe with long-acting insulins, but its absorption may be delayed and prolonged.

Insulin aspart

Insulin aspart is another fast acting insulin analogue. It is prepared through recombinant DNA technology by substituting the amino acid B28, which is normally proline, with an aspartic acid residue. This substitution does not alter the drug binding to insulin receptor, but increases charge repulsion which prevents the formation of hexamers. The onset of action is approximately 15 minutes, the peak action is reached in 45–90 minutes, and the duration is 3–5 hours. The pharmacological properties and usage of insulin aspart is similar to those of insulin lispro. Injection of a mixture containing 30% insulin aspart and 70% insulin aspart protamine delays the onset (~30 min) and provides a long duration of action (~ 24 hours).

Insulin glulisine

Insulin glulisine is a rapid-acting insulin analogue that differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid.

2. Intermediate Acting Insulin Preparations

Intermediate acting insulins are formulated so that they dissolve more gradually when administered subcutaneously. Their duration of action is thus longer than the rapid acting insulins.

Isophane insulin

Isophane insulin, also called Neutral Protamine Hagedorn (NPH) insulin, is a suspension of crystalline zinc insulin combined with protamine in a phosphate buffer at neutral pH. Protamine, a positively charged polypeptide, retards the absorption of insulin and thereby prolongs its duration of action. After SC administration, onset of action occurs within 2 hours, peak effect occurs at 2 to 8 hours, and duration of action is 16-12 hours in cats and up to 18 hours in dogs. Twice daily administration of NPH is usually necessary because it causes wide fluctuation in blood glucose. Some practitioners do not recommend it for cats. If two injections a day are used, two meals of equal calories are given at the same time of insulin administration. Clinical signs and serial blood glucose determinations are used to monitor therapy. In general, NPH is the initial insulin of choice in dogs.

A number of premixed insulins having a fixed ratio of NPH insulin to regular insulin are also available for providing faster action and allowing more accurate dosing and better blood sugar level control. These preparations consist of 70% NPH and 30% regular insulin (70/30) and 50% NPH and 50% regular insulin (50/50). Other premixed preparations of 90/10 and 80/20 are also available in some countries.

Dose

Isophane insulin (NPH insulin)

Dogs : 0.5 unit/kg, SC, 1 to 2 times daily.
Cats : 1 – 3 units (total), SC, 1 to 2 times daily.

Lente insulin

Lente insulin (Insulin zinc suspension, IZS) is a mixture of 30% semi-lente insulin (amorphous insulin) with 70% ultra-lente insulin (crystalline insulin) in an acetate buffer. This combination minimises the solubility of insulin and provides a sustained action, making it an intermediate acting preparation. The onset of action, peak effect and duration of action following SC administration are similar to those of isophane insulin. It has proved a useful preparation in the long-term management of diabetes mellitus in dogs and cats. This agent should not be used in the case of diabetic coma or emergency situations.

Dose

Dogs & cats : 0.3 – 1.3 unit/kg, SC, 1 to 2 times daily.

3. Long Acting Insulin Preparations

Long-acting insulins have very slow onset of action and a prolonged, relatively flat peak of action. These insulins have been advocated to provide a low basal concentration of insulin throughout the day.

Protamine zinc insulin

Protamine zinc insulin (PZI) complex was originally developed in year 1936 in which insulin was combined with zinc and an excess of protamine in a buffered aqueous solution was added such that large zinc insulin crystals with poor solubility are formed. The poor solubility of the complex prolongs the absorption time of insulin

after subcutaneous administration and provides a slower onset of activity, but a longer duration of action. When injected SC, the action of PZI begins in about 4 hours, peaks in 16-18 hours, and lasts for up to 36 hours. Because of faster metabolism in some cats, PZI may be used twice daily. Protamine zinc insulin is rarely used today in human medicine because of its unpredictable and prolonged course of action, but is convenient for use in veterinary medicine for long term-management of diabetes mellitus in small animals. It is used also in hyperlipaemia in ponies. The PZI should not be used to treat diabetic coma or in emergency situations.

Dose

Dogs & cats : 0.2 – 1 unit/kg, SC, 1 to 2 times daily.

Ponies & horses : 0.15 unit/kg, SC, 2 times daily in combination with carbohydrate treatment.

Ultra-lente insulin

Ultra-lente insulin (Extended insulin zinc suspension) is a large crystalline form of insulin with high zinc content in acetate buffer. It has particle size of 10 to 40 µm and is relatively insoluble in extracellular fluids; hence is absorbed very slowly producing a slow onset of action and a long-lasting hypoglycaemic effect. After SC administration, the onset of action starts at 4 hours, peaks at 10-30 hours and produces duration of about 36 hours (comparable with PTZ). The long-half life of ultra-lente insulin makes it difficult to determine the optimal dosage, since several days of treatment are required before a steady state concentration of circulating insulin is achieved. It is useful in the long-term management of diabetes mellitus in cats and hyperlipaemia in ponies. Ultralente insulin should not be used in the case of diabetic coma or emergency situations.

Dose

Dogs & cats : 0.5 – 1 unit/kg, SC, 1 to 2 times daily.

Insulin glargine

Insulin glargine is a long-acting basal insulin analogue obtained by the recombinant DNA technology. It has substitution of glycine for asparagine at A21 and two arginines added to the carboxy terminal of B chain. Insulin glargine is formulated at an acidic pH 4, where it is completely water soluble. However after subcutaneous injection, the insulin at physiological pH (approximately 7.4) forms microcrystals (called insulin hexamers), which then slowly dissociate into insulin monomers, the functional and physiologically active unit of insulin. This gradual process ensures that small amounts of insulin are released into the body continuously, giving an almost peakless profile for about 24 hours. Therefore, pharmacokinetically insulin glargine resembles basal insulin secretion of non-diabetic pancreatic beta cells. Insulin glargine thus has advantage over standard NPH insulin in providing a sustained level of insulin and in reducing the risk of severe nocturnal hypoglycaemia.

Insulin glargine has largely been approved for use in human patients. It is given once daily to help control the blood sugar level in diabetic patients. Unlike some other long-acting insulins, insulin glargine must not be diluted or mixed with other insulin or solution in the same syringe.

Insulin detemir

Insulin detemir is another long-acting human insulin analogue prepared for maintaining the basal level of insulin. It contains a fatty acid (myristic acid) bound to the lysine amino acid at position B29. It is quickly absorbed after which it binds to albumin in the blood through the fatty acid at position B29. It then slowly dissociates from this complex providing a long duration of action. Similar to insulin glargine, it provides a sustained level of insulin and thus reduces risk of hypoglycaemia. Insulin detemir comes as a solution (liquid) for once daily SC injection. Insulin detemir should not be mixed or diluted with other insulin products.

4. Ultra-Long Acting Insulin Preparations

Insulin degludec

Insulin degludec is a new basal insulin analogue designed for providing ultra long duration of action. After SC injection, it forms soluble multihexamer assemblies from which insulin monomers are slowly released resulting in an ultra-long action profile. Its action lasts for about 40 hours, unlike the 18 to 26 hours provided by current marketed long-acting insulins such as insulin glargine and insulin detemir. Insulin degludec is currently in Phase III clinical trials

Table 53.2: Important insulin preparations.

Preparation	Appearance	Buffer*	Added protein	Zinc Content (mg/100 U)
Rapid/Short Acting Insulin Preparations				
Regular insulin (Soluble insulin)	Clear	None	None	0.01 – 0.04
Semi-lente insulin	Cloudy	Acetate	None	0.2 – 0.25
Insulin lispro	Clear	Phosphate	None	0.02
Insulin aspart	Clear	Phosphate	None	0.02
Insulin gluisine	Clear	None	None	None
Intermediate Acting Insulin Preparations				
Isophane insulin (NPH insulin)	Cloudy	Phosphate	Protamine	0.016 – 0.04
Lente-insulin	Cloudy	Acetate	None	0.2 – 0.25
Long-Acting Insulin Preparations				
Protamine zinc insulin (PZI)	Cloudy	Phosphate	Protamine	0.2 – 0.25
Ultra-lente insulin	Cloudy	Acetate	None	0.2 – 0.25
Insulin glargine	Clear	None	None	0.03

* Most insulin preparations are supplied at pH 7.1 – 7.4; Insulin glargine is supplied at a pH of 4.0.

Mixtures of Insulin Preparations

Combination of insulin preparations is often used in human medicine to induce biphasic effect with prompt onset and intermediate duration of action. This can be achieved by combining regular (soluble) insulin with intermediate or long-acting insulins in different proportions. Regular insulin is used to assist in assimilation of carbohydrate boluses during the day, whereas intermediate or long-acting insulin supplies a basal insulin concentration throughout the day. Insulin combinations are less frequently used in diabetic animals because meticulous glucose control is seldom achieved or necessary in veterinary medicine. Insulin combinations may be useful in the long-term management of diabetic patients with insulin resistance and in animals having a tendency toward ketosis.

Insulin Syringes and Insulin Delivery System

Insulin is available commercially in 40, 100 and 500 units/ml concentration, which are designated as U-40, U-100 and U-500, respectively. One unit of insulin is approximately equivalent to 36 µg of insulin. Syringes are available for the corresponding insulin concentrations and should be used for the same i.e. only U-100 syringe should be used to measure U-100 insulin and only U-40 syringe should be used to measure U-40 insulin. The U-100 syringes are manufactured as low dose (0.3 ml and 0.5 ml) and 1 ml capacities and U-40 syringes are available only as 1 ml capacity. In dogs and cats which require extremely small dosages of insulin, the U-100 insulin preparations may be diluted with diluent specifically designed by the manufacturer for that insulin preparation. Special care must be taken in such cases to administer the correct dose of diluted insulin with an ordinary insulin syringe. If a 1: 10 dilution of insulin is prepared, a full 1 ml U-100 syringe will contain only 10 Units, a full 0.5 ml syringe will contain 5 units, and full 0.3 ml syringe only 3 units.

Recently some newer insulin delivery devices have been developed and have gained popularity in human medicine. These include jet injections (deliver insulin SC without using a needle), pen injections (pen sized syringes which use insulin cartridges for SC injection through a needle), insulin pumps (portable insulin devices connected to SC placed cannula), and implantable pumps (percutaneously refillable insulin reservoir). The cost and the cumbersome nature of these devices have restricted their use in animals.

B. Insulin Secretagogues

Insulin secretagogues are a group of chemically diversified agents which cause release of insulin from its storage vesicles. These agents act by different mechanisms, but blocking of K_{ATP} channel of the pancreatic beta cells is the most important and effective mechanism of insulin release. The insulin secretagogues are used mainly in human medicine to lower blood glucose in patients with non-insulin dependent diabetes mellitus (NIDDM). These agents have limited applications in veterinary medicine because most cases of diabetes mellitus in dogs and cats require insulin for control.

1. Sulphonylureas /Oral Antidiabetic Drugs

Sulphonylureas are chemically related to the sulphonamides, but they have no antibacterial activity. All members of this class of drugs are substituted arylsulphonylureas, which have similar mechanism of action and pharmacological profile with differences in potency and duration of action. Sulphonylureas are used almost exclusively in diabetes mellitus type 2. Sulphonylureas are ineffective where there is absolute deficiency of insulin production such as in type 1 diabetes or post-pancreatectomy.

Mechanism of Action

Sulphonylureas act mainly on the beta cells of the islets and stimulate insulin secretion. These agents are thought to exert their effect by blocking ATP-dependent K^+ (K_{ATP}) channel on the cell membrane of pancreatic beta cells. This decreases potassium exit from the β-cells, thus causing the electric potential over the membrane to become more positive. The reduced potassium conductance and subsequent membrane depolarization activates the voltage sensitive calcium channels. Opening of calcium channels promote influx of Ca^{++}, which in turn evokes exocytosis of storage granules resulting in insulin release. Synthesis of insulin, however, is not increased. Sulphonylureas are also reported to reduce serum glucagon levels.

Extrapancreatic effects of sulphonylureas include enhancement of peripheral sensitivity to insulin (increased binding of insulin to target tissue and receptors) and decreased production of hepatic basal glucoses.

a. First Generation Sulphonylureas

Chlorpropamide

Chlorpropamide is a prototype first generation oral sulphonylurea antidiabetic agent. It occurs as a white crystalline powder that is practically insoluble in water. In veterinary medicine, chlorpropamide is used occasionally in non-insulin dependent diabetes mellitus (NIDDM) and also diabetes insipidus.

Pharmacological effects

Chlorpropamide and other sulphonylureas lower blood glucose level in both diabetic (only Type 2) and non-diabetic cases. They also lower the plasma free fatty acid levels. Like other sulphonylureas, chlorpropamide acts to increase the secretion of insulin. Therefore, it is effective only in diabetes with some (at least 30%) functional beta cells and is without effect in pancreatic beta-cell destruction (Type 1 diabetes or IDDM) or in pancreatectomised animals. Chlorpropamide has also an antidiuretic effect, presumably by stimulating secretion of vasopressin and/ or by potentiating vasopressin's effect on renal tunnels. Chlorpropamide causes about 20-50% reduction in urine output in dogs at 1-2 week intervals of therapy (see also Chapter 49).

Pharmacokinetics

Chlorpropamide is well absorbed after oral administration and appears in blood within 1–2 hours. It is highly (~90%) bound to plasma proteins and metabolised in the liver. Elimination occurs mainly in urine both as metabolites and unchanged drug. It is also excreted in milk and may cross placenta. Elimination half-life in humans is about 30-36 hours. Elimination of chlorpropamide is enhanced in alkaline urine and decreased in acidic urine.

Side effects/Adverse effects

Chlorpropamide is usually well tolerated in recommended doses. Hypoglycaemia is the most common adverse effect noted with chlorpropamide. Rarely, anorexia, vomiting, diarrhoea and other GI signs may develop. In human beings, chlorpropamide in addition causes cholestatic jaundice, dilutional hyponatraemia (sensitises the kidneys to vasopressin) and intolerance to alcohol (flushing and a disulfiram like reaction). Overdosage in all species may produce profound hypoglycaemia.

Contraindications and precautions

Chlorpropamide and other sulphonylureas are contraindicated in diabetic coma, ketoacidosis, ketosis and various hypoglycaemic conditions. Chlorpropamide and other sulphonylureas should not be used in pregnancy because they cross the placenta and stimulate foetal β-cells to release and deplete insulin. Chlorpropamide should be used with caution in patients with hepatic or renal impairment or when fluid retention is present. Continuous use of chlorpropamide may lead to exhaustion of islet β-cells with failure of therapy.

Drug interactions

Several compounds augment the hypoglycaemic effect of chlorpropamide and other sulphonylureas through pharmacokinetic and pharmacodynamic interactions. Non-steroidal anti-inflammatory drugs (NSAIDs), coumarins, mono-amine oxidase inhibitors, some antibacterial (e.g. sulphonamides, trimethoprim and chloramphenicol), some antifungal drugs (e.g. miconazole and fluconazole) and probenecid have all been reported to produce severe hypoglycaemia when given with chlorpropamide and other sulphonylureas. This mainly occurs due to competition for the plasma protein binding, interference with metabolising enzymes or excretion of drugs (see also Table 53.3). Agents which decrease the action of sulphonylureas include diuretics (e.g. thiazides and loop diuretics) and corticosteroids.

Clinical uses

In veterinary medicine, chlorpropamide is used in the adjunctive treatment of non-insulin dependent diabetes mellitus (NIDDM) in small animals. It is also used in the treatment of diabetes insipidus in dogs and cats.

Dose

For NIDDM

Dogs : 250 – 500 mg (total), PO, 2 times daily with food. Dose may be adjusted to produce normoglycaemia.

Tolbutamide

Tolbutamide is another first generation potassium channel blocker, sulphonylurea oral hypoglycaemic drug. Similar to other sulphonylureas, tolbutamide acts by stimulating the secretion of insulin from the pancreas. This drug is occasionally used in the management of type II diabetes. As tolbutamide is an insulin secretagogue, it is not effective in the management of type I diabetes. The pharmacological and side/adverse effects of tolbutamide are in general similar to those of chlorpropamide.

Dose

For NIDDM

Tolbutamide

Dogs : 20 –100 mg/kg, PO, daily. Dose should be adjusted to produce normoglycaemia.

Other first generation sulphonylureas

Tolazamide, carbutamide and acetohexamide are other first generation sulphonylureas. These drugs are used rarely in veterinary medicine for the control of non-insulin dependent diabetes mellitus.

b. Second Generation Sulphonylureas

The second generation of sulphonylurea antidiabetic agents are considerably more potent than the older first generation agents. They all contain the sulphonylurea moiety and have both pancreatic and extra-pancreatic effects, but different substitutions result in differences in potency, pharmacokinetics and duration of action.

Glipizide

Glipizide (Glydiazinamide) is a potent second generation sulphonylurea that closely resembles the first generation sulphonylurea acetohexamide. In veterinary medicine, glipizide is used mainly in cats for the treatment of non-insulin dependent diabetes mellitus.

Pharmacological effects

The pharmacological effects of glipizide are similar to those of chlorpropamide, but it is about 15-20 times more potent than the chlorpropamide. Glipizide stimulates insulin secretion in cats with functional β-cells. It causes a significant increase in serum insulin concentration and a corresponding fall in serum glucose concentration within 10 minutes of its oral administration.

Pharmacokinetics

Glipizide is rapidly and almost completely absorbed after oral administration. It is highly bound to plasma proteins and metabolised in liver. In cats, the effect peaks in about 15 minutes and lasts for about 1 hour. In humans, half-life of glipizide is about 2–4 hours. Glipizide is excreted primarily as metabolites in the urine.

Side effects/Adverse effects

Adverse effects to glipizide therapy in diabetic cats (~10%) include hypoglycaemia, vomiting, cholestasis and increased hepatic

Table 53.3: Drug interactions with sulphonylurea antidiabetic drugs.

Mechanism	Interacting Drugs
Sulphonylurea Efficacy Increased	
1. Displacement from plasma protein binding.	Sulphonamides, phenylbutazone, salicylates, sulfinpyrazone, clofibrate and warfarin.
2. Inhibition of biotransformation	Chloramphenicol, dicoumarol and cimetidine.
3. Inhibition of excretion	Probenecid and allopurinol.
4. Synergistic pharmacological actions	Beta-adrenoceptor blockers (e.g. propranolol), levodopa, mono-amine oxidase inhibitors and theophylline.
Sulphonylurea Efficacy Reduced	
1. Enzyme induction	Barbiturates (e.g. phenobarbital), rifampicin and phenytoin.
2. Induction of hypoglycaemia	Diuretics (e.g. thiazides and furosemide), glucocorticoids, thyroxine and beta-adrenoceptor agonists.

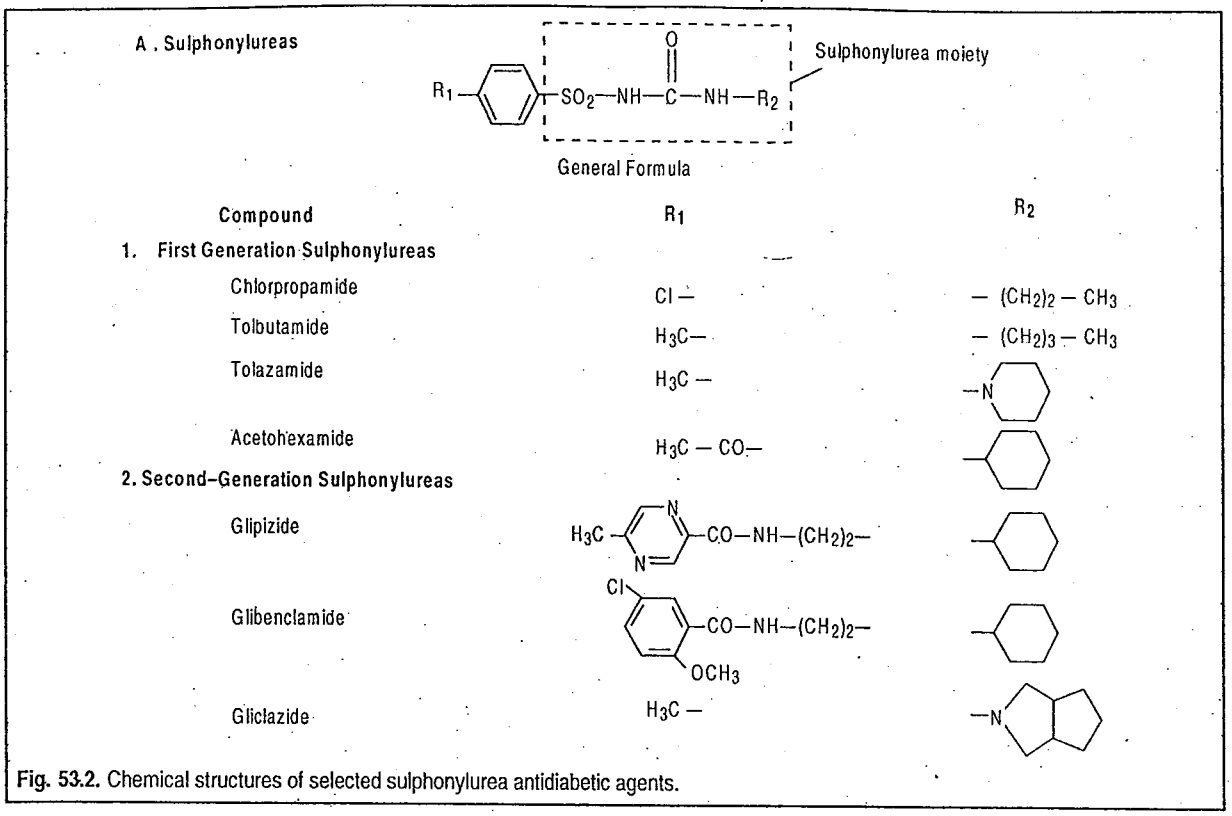


Fig. 53.2. Chemical structures of selected sulphonylurea antidiabetic agents.

enzyme activities. In man, glipizide therapy in addition may produce rashes, pruritus, bone marrow depression and blood dyscrasias. Overdosage of glipizide may produce profound hypoglycaemia in both man and cats.

Contraindications and precautions

Contraindications of glipizide are similar to those of chlorpropamide. Safe use during pregnancy has not been established but glipizide has found to be mildly foetotoxic in rats. Glipizide should not be used in weak or ketonuric cats when absolute deficiency is likely and exogenous insulin administration is required. Some veterinarians consider glipizide not suitable for use in dogs.

Drug interactions

In general, drug interactions occur mainly due to competition for plasma proteins bindings and interference in biotransformation and/or excretory processes (Table 53.3).

Clinical uses

Glipizide is considered suitable for the treatment of non-insulin dependent diabetes mellitus in cats. It provides a viable alternative to conventional insulin therapy in approximately 50% of cats with naturally occurring diabetes. Response to glipizide therapy or lack thereof usually is evident within the first 3 to 4 weeks of treatment. The existence of residual β-cells function is necessary for response to glipizide therapy.

Dose

Dogs & cats : 0.25 – 0.5 mg/kg, PO, 2 times daily.

Glibenclamide

Glibenclamide (Glyburide) is a general sulphonylurea antidiabetic agent, which is about 1.5 times more potent than glipizide. Its pharmacological profile is similar to those of glipizide and is used occasionally in small animals for the treatment of non-insulin dependent diabetes mellitus.

Table 53.4: Comparative potency of selected sulphonylurea antidiabetic drugs.

Drug	Potency
First generation	
Tolbutamide	1
Acetohexamide	2.5
Tolazamide	5
Chlorpropamide	6
Second generation	
Glipizide	100
Glibenclamide	150

Dose

Dogs & cats : 0.2 mg/kg, PO, 2 times daily with meal.

Other second generation sulphonylureas

Gliclazide, gliquidone, glyclopyramide and glimepiride are other second-generation sulphonylurea which are used occasionally in human medicine for the treatment of diabetes mellitus type 2. Glimepiride is sometimes also classified as the third-generation sulfonylurea, due to its very high potency.

2. Non-sulphonylurea Secretagogues

a. Meglitinides

Meglitinides, also called glinides, are a class of anti-diabetic drugs used to treat diabetes type 2. These drugs bind to an ATP-dependent K^+ (K_{ATP}) channel on the cell membrane of pancreatic beta cells in a manner similar to sulphonylureas, but at a separate binding site. The drugs are currently not used in animals.

Mitiglinide

Mitiglinide is an orally acting insulin secretagogue that acts by inhibiting ATP-dependent K^+ (K_{ATP}) channel in the pancreatic beta-cells. Closure of potassium channels causes depolarization which stimulates calcium influx through voltage-gated calcium channels. High intracellular calcium subsequently triggers the exocytosis of insulin granules. Mitiglinide has been approved for the treatment of type 2 diabetes.

Repaglinide

Repaglinide is a meglitinide analogue oral hypoglycaemic drug designed to treat Type 2 diabetes. Similar to meglitinide, it is thought to stimulate insulin secretion by binding to and blocking ATP-sensitive K^+ (K_{ATP}) channels. Repaglinide induces rapid onset, but short-lasting insulin release. Because of short-lasting action, it may have a lower risk of serious hypoglycaemia. Side/adverse effects are mild and may include dyspepsia, headache, arthralgia, weight gain in human patients. Like other insulin secretagogues, repaglinide is indicated only in type 2 diabetes mellitus. It is often used as an alternative to sulphonylureas, or to supplement long-acting insulins. It should be avoided in liver diseases.

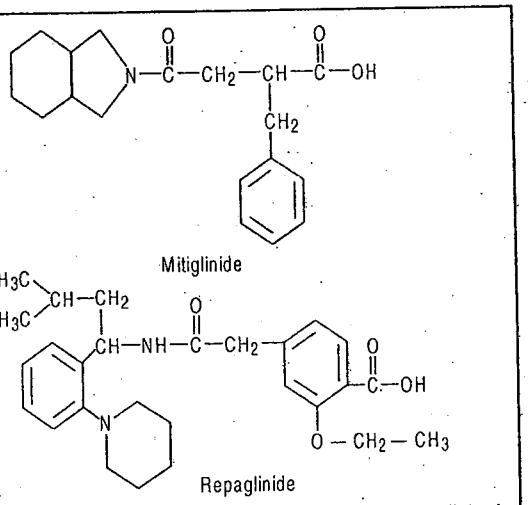


Fig. 53.3. Chemical structures of selected meglitinide anti-diabetic drugs.

Nateglinide

Nateglinide is another orally active anti-diabetic drug belonging to the class of mitiglinide drugs. It has a rapid onset and shorter duration of hypoglycaemic action than repaglinide. Episode of hypoglycaemia are less frequent than sulphonylureas.

b. Glucagon-Like Peptide-1 Analogues

Glucagon-like peptide-1 analogues are a new class of anti-diabetic drugs approved for treatment of type 2 diabetes. These drugs mimic the action of Glucagon-like peptide-1 (GLP-1), an incretin (gut hormone) involved in pancreatic insulin secretion after ingestion of food. It also prevents glucagon release and gastric emptying. However, GLP-1 cannot be used as a drug due to poor stability. Some GLP-1 analogues have been synthesized and are currently available for use. These include recently approved exenatide and liraglutide. Albiglutide and taspoglutide are currently in clinical trials.

Exenatide

Exenatide is a synthetically prepared 39-amino-acid peptide, which is structurally similar to exendin-4, a hormone found in the saliva of the Gila monster. It displays biological properties similar to those of human glucagon-like peptide-1 (GLP-1), an incretin involved in regulation of glucose metabolism and insulin (Incretins are a group of gastrointestinal hormones which cause an increase in the amount of insulin released from the beta cells of the islets of Langerhans after eating, even before blood glucose levels become elevated). Similar to GLP-1, exenatide binds to and activates GLP-1 receptors in pancreatic beta cells and enhances secretion of insulin. Like GLP-1, it also increases insulin-sensitivity in both alpha cells and beta cells, enhances beta cells mass and insulin gene expression, and decreases glucagon secretion from the pancreas. Additionally exenatide helps slow down gastric emptying and thus decreases the rate at which meal-derived glucose appears in the bloodstream.

Exenatide has been approved as adjunctive therapy to improve glucose control in patients with type 2 diabetes mellitus. Since the major action of this drug is to enhance the release of endogenous insulin from the pancreas, exenatide is not for use in type 1 diabetes. The main side/adverse effects of exenatide use are gastrointestinal disturbances including nausea, vomiting, diarrhoea and indigestion. Other side effects include dizziness and headache. Unlike sulphonylureas and other insulin secretagogues, exenatide is administered by SC injection.

Liraglutide

Liraglutide is an acylated human glucagon-like peptide-1 (GLP-1) receptor agonist, with a 97% amino acid sequence homology to endogenous human GLP-1. Similar to exenatide, it controls meal-related hyperglycaemia by increasing insulin secretion, delaying gastric emptying, and suppressing prandial glucagon secretion. Liraglutide is administered by SC injection for the treatment of type 2 diabetes.

c. Dipeptidyl Peptidase-4 Inhibitors

Inhibitors of dipeptidyl peptidase 4, also called DPP-4 inhibitors or gliptins, are a novel class of oral hypoglycaemic drugs. These drugs bind to and inhibit dipeptidyl peptidase-4, an enzyme involved in degradation of incretins such as glucagon-like peptide-1 (GLP-1). These drugs have been approved for treatment of type 2 diabetes.

Sitagliptin

Sitagliptin is an oral antidiabetic drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. It was approved for clinical use in year 2006 and works by competitively inhibiting the enzyme dipeptidyl peptidase 4 (DPP-4), which is involved in biotransformation of incretins such as GLP-1. By preventing inactivation of GLP-1 and other gastrointestinal hormones, sitagliptin indirectly enhances the secretion of insulin and suppresses the release of glucagon by the pancreas. Compared with other hypoglycaemic drugs, sitagliptin and other DPP-4 inhibitors have shown lesser incidence of hypoglycaemia. Sitagliptin is recommended for treatment of type 2 diabetes in human medicine, either alone or in combination with other oral antihyperglycaemic agents such as metformin or a thiazolidinedione. Nausea, vomiting and common cold-like symptoms are important side/adverse effects observed with sitagliptin. There are no reports of sitagliptin use in small animals.

Other drugs

Some other dipeptidyl peptidase 4 inhibiting drugs include alogliptin, vildagliptin, saxagliptin, linagliptin and dutogliptin. Vildagliptin, saxagliptin and alogliptin have been approved for treatment of type 2 diabetes, whereas linagliptin and dutogliptin are in the clinical trials.

C. Insulin Sensitizers

Insulin sensitizers are a group of drugs which enhance binding of insulin to its receptors and stimulate insulin mediated glucose disposal. These drugs include two groups of oral anti-diabetic drugs – biguanides and thiazolidinediones.

1. Biguanides

Biguanides are orally active antihyperglycaemic agents. Biguanides do not affect the release of insulin, unlike insulin secretagogues such as sulphonylureas and meglitinides. Therefore, they are effective not only in Type 2 diabetics but also in Type 1 diabetic patients in concert with insulin therapy. They are mainly used in human medicine and have limited importance in veterinary medicine.

Mechanism of action

In contrast to sulphonylureas, the action of biguanides is extrapancreatic and does not depend of the presence of endogenous insulin in the body. They stimulate mainly the peripheral utilisation of glucose and suppress hepatic gluconeogenesis and glucose output from the liver. They also enhance binding of insulin to its receptors and stimulate insulin mediated glucose disposal. Biguanides inhibit intestinal absorption of glucose, other hexoses, amino acids and vitamin B_{12}. Although they do not cause release of insulin, it has been suggested that presence of some insulin is essential for their action.

Metformin

Metformin is an orally active biguanide that is occasionally used in veterinary medicine to resolve disorders associated with insulin resistance. It neither causes insulin release from pancreas nor causes hypoglycaemia, even in larger doses. Metformin is absorbed mainly from the small intestine. It does not bind to plasma proteins. In humans, it is excreted mainly unchanged in urine and has a half-life of 1.5 to 4.5 hours.

Adverse effects reported in man include diarrhoea, abdominal discomfort, nausea and anorexia. Lactic acidosis is a potentially severe adverse effect of the biguanide antidiabetic drugs, but it occurs rarely with metformin. In human medicine, metformin is used alone or in combination with a sulphonylurea to improve glycaemic control and lipid concentration in patients who respond poorly to diet or a sulphonylurea alone. In veterinary medicine, metformin may be used in hyperglycaemic cats, but it requires further investigation.

Dose

Cats : 2 mg /kg, PO, 2 times daily. Follow up studies are required.

Phenformin

Phenformin is an oral biguanide antidiabetic agent that is rarely used in therapeutics. Phenformin produces high incidence of ketouria and lactic acidosis in human patients hence it has been withdrawn from therapy in many countries.

2. Thiazolidinediones

The thiazolidinediones belong to a new class of antihyperglycaemic agents which appear to augment insulin action in insulin-resistance animals. Pioglitazone, rosiglitazone, englitazone, ciglitazone and troglitazone are important members of the group.

Mechanism of action

Thiazolidinediones act by a novel mechanism to decrease insulin resistance in diabetic patients. These drugs are selective agonists for the nuclear peroxisome proliferator-activated receptor-gamma (PPARγ), which enhances the transcription of several insulin responsive genes. They modulate transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in muscle, adipose tissue and liver. The thiazolidinediones help to reverse insulin resistance also by stimulating expression of transporter GLUT-4, which facilitates entry of insulin into the muscles and fat. Hepatic gluconeogenesis is also suppressed by these agents. Thus, thiazolidinediones do not increase insulin release but enhance insulin action and tissue utilisation of glucose.

Pioglitazone

Pioglitazone is an anti-diabetic belonging to the drug class of the thiazolidinediones. Pioglitazone, like the other thiazolidinediones, works by activating peroxisome proliferator-activated receptors (PPARs). Pioglitazone has been approved for use in human diabetic patients for the treatment of diabetes mellitus type 2. It is ineffective and possibly harmful in diabetes mellitus type 1 and diabetic ketoacidosis. Important side/adverse effects related to pioglitazone in human patients are plasma volume expansion, oedema, weight gain, myalgia, headache and mild anaemia. It is used both in monotherapy and in combination with a sulphonylurea, metformin or insulin.

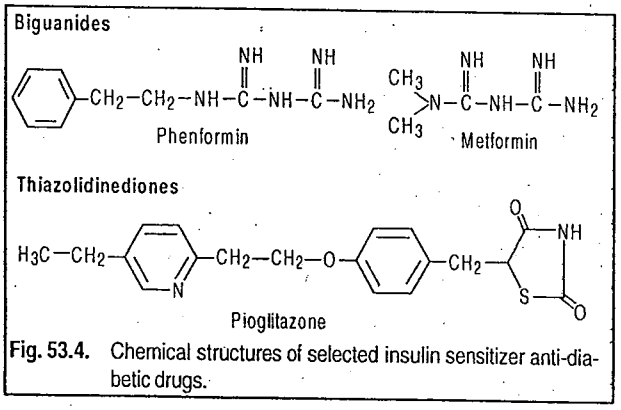


Fig. 53.4. Chemical structures of selected insulin sensitizer anti-diabetic drugs.

D. Miscellaneous Drugs

1. Alpha-Glucosidase Inhibitors

Acarbose

Acarbose is a complex oligosaccharide of bacterial origin that is occasionally used to treat type 2 diabetes mellitus. It acts by competitively inhibiting the action of intestinal brush border enzyme α-glucosidase, which digests carbohydrates and releases glucose from larger carbohydrates. It also competitively inhibits glucoamylase and sucrose but has weak effects on pancreatic

α-amylase. Inhibition of these enzymes decreases degradation of complex carbohydrates into absorbable monosaccharides and thus slows the absorption of carbohydrates. It reduces post-prandial plasma glucose levels in insulin dependent and non-insulin dependent diabetes mellitus. The drug is not absorbed, so systemic effects are not common. Flatulence, soft stools and diarrhoea may occur due to the osmotic effect and bacterial fermentation of non-digested carbohydrates in colon.

Acarbose is recommended for humans primarily to reduce post-prandial glucose fluctuations and to improve glycaemic stability when response to traditional oral antidiabetic drugs is insufficient. If a patient administered acarbose suffers from a bout of hypoglycaemia, the patient should be given something containing monosaccharides such as glucose tablets. It has been studied in dogs and found suitable in some cases.

Dose

Dogs : 100 – 200 mg (total), PO with each meal.

Miglitol

Miglitol is an oral anti-diabetic drug that acts by inhibiting the intestinal brush border enzyme α-glucosidase. Similar to acarbose, this decreases patient's ability to breakdown complex carbohydrates into glucose. It actions and pharmacological effects are similar to those of acarbose. However, in contrast to acarbose, miglitol is systemically absorbed and excreted largely unmetabolized in urine. It is primarily used in diabetes mellitus type 2.

Voglibose

Voglibose is the latest alpha-glucosidase inhibitor introduced for use in diabetic type 2 patients. The pharmacological effects of voglibose are similar to those of acarbose, but it is reported to possess lesser incidence of side effects.

2. Amylin Analogues

Pramlintide

Pramlintide is a relatively new antidiabetic drug mainly used in adjunct therapy. It is a synthetic analogue of amylin, a small peptide hormone secreted by pancreatic β-cells at the same time as insulin. Similar to insulin, amylin contributes to glycemic control and is deficient in diabetic patients. By augmenting endogenous amylin, pramlintide aids in the absorption of glucose by slowing gastric emptying, promoting satiety via hypothalamic receptors, and inhibiting inappropriate secretion of glucagon, a catabolic hormone that opposes the effects of insulin and amylin. Pramlintide has been approved for use in both type 1 and type 2 diabetes, mainly in adjunct therapy. It is administered by SC injection just before a meal and attenuates post-prandial hyperglycaemia.

3. Sodium-Dependent Glucose Co-Transporter Inhibitors

Sodium-dependent glucose co-transporters (SGLT) are a family of glucose carriers specifically expressed in the intestinal mucosa of small intestine (SGLT-1) and proximal tubule of nephron (SGLT-2). The SGLT-1 is responsible for absorption of glucose from the intestine, while SGLT-2 is responsible for reabsorption of glucose from renal tubules. At least ten SGLT inhibitors are in clinical development; most of the currently developed SGLT inhibitors are selective for SGLT-2, a minority is directed at SGLT-1. Inhibitors of SGLT-1 suppress absorption of glucose from the gut, while SGLT-2 inhibitors block the reabsorption of glucose from the renal filtrate.

Canagliflozin and Dapagliflozin

Canagliflozin and dapagliflozin are two sodium-glucose transport proteins (SGLT-2) inhibitors currently in clinical trials for use in diabetes type 1 and types 2. They block about 90% of the glucose reabsorption in the kidney and cause blood glucose to be eliminated through the urine. These SGLT inhibitors are designed to be complementary approaches to traditional anti-diabetics intervening with glucose metabolism.

4. Other drugs

Guar gum

Guar gum is a soluble dietary fibre obtained from Indian cluster beans (guar). It is available in granular form and is a high molecular weight polysaccharide (a galactomannan). On oral administration, it forms a viscous gel in the intestine and decreases carbohydrate absorption, thereby reducing blood glucose levels. It is used in human medicine in diabetes mellitus as an adjunct to insulin or oral antidiabetic therapy.

Glucomannan

Glucomannan is a powdered extract obtained from tubers of konjac, a plant of the genus *Amorphophallus*. The component sugars are β-(1>4)-linked D-mannose and D-glucose in a ratio of 1.6:1. Glucomannan is a soluble fibre and as such swells in stomach on ingestion by absorbing water. It is claimed to reduce appetite, blood glucose, serum lipids and relieve constipation. Products containing glucomannan are available as nutritional supplements for constipation, obesity, high cholesterol and type-2 diabetes.

II. DRUGS USED IN EXCESS OF INSULIN

Glucagon

Glucagon is a protein hormone synthesized and secreted from alpha cells of the islets of Langerhans. Its physiological effects are opposite to those of insulin as it is released when blood sugar (glucose) levels fall too low. The physiology and pharmacology of glucagon has been discussed separately in the subsequent text in the present chapter.

Octreotide

Octreotide is synthetic analogue of somatostatin. It is commonly used in human medicine for the treatment of certain cancers such as tumour of vasoactive intestinal peptide (VIP-oma) and carcinoid. Octreotide is also used to treat acromegaly due to excess secretion of growth hormone. In dogs octreotide acetate has been used in the treatment of some insulinomas and gastrinomas. The pharmacology of octreotide has been discussed in Chapter 49.

Dose

For treatment of insulinoma

Dogs : 10 – 20 µg/kg, SC, 2 to 3 times daily.

For treatment of gastrinoma

Dogs : 20 – 60 µg/kg, SC, 3 times daily.

Diazoxide

Diazoxide is a non-diuretic benzothiadiazine, used mainly as a vasodilator in the treatment of acute hypertension or malignant hypertension. It is also considered useful in the management of excessive secretion of insulin in disease states such as insulinoma or congenital hyperinsulinism. It decreases insulin release from beta cells by increasing permeability to potassium, which causes hyperpolarization of membrane. It also promotes hepatic glycogenolysis and decreases glucose uptake in the liver.

Dose

Small animals : 10 mg/kg, PO, divided into two daily doses. The dose may be gradually increased, but should not exceed 40 mg/kg/day.

GLUCAGON

Glucagon is a protein hormone produced and secreted by the α-cells of the pancreatic islets of Langerhans. It has a close relationship with insulin in the control of glucose metabolism and has effects opposite to those of insulin.

Synthesis, Release and Fate

Synthesis and release: Glucagon is a single-chain polypeptide composed of 29 amino acids with a molecular weight of 3485. Unlike other polypeptide hormones, the structure of glucagon is identical in most mammals including human beings, cattle, pigs and rats. Glucagon shows significant homology with several other polypeptide hormones including secretin, vasoactive intestinal peptide (VIP) and gastrointestinal inhibitory polypeptide (GIP). Like other polypeptide hormones, glucagon is synthesised in the endoplasmic reticulum as a part of a precursor molecule, packaged in Golgi apparatus with final processing occurring in secretory granules, and released by exocytosis. In addition to pancreas, glucagon may also be synthesised by gastric cells called gut glucagon. Small intestine produces an immunologically similar molecule called glicentin.

Metabolism: Glucagon is metabolized mainly by liver and kidneys. Proteolytic removal of the amino-terminal histidine residue leads to loss of biological activity. Glucagon has a plasma half-life of about 5 minutes.

Regulation of Secretion

The secretion of glucagon is regulated by several factors including glucose (most important), insulin, amino acids and fatty acids. Glucagon release is stimulated during hypoglycaemia and inhibited during hyperglycaemia. Insulin inhibits glucagon release; thus in insulin insufficiency as in diabetes mellitus, the plasma glucagon concentrations are elevated. Gastrointestinal peptides such as cholecystokinin, gastrin and gastric inhibitory polypeptide and catabolic hormones such as catecholamines, growth hormone and glucocorticoids stimulate release of glucagon, while somatostatin inhibits glucagon release. Increase in free fatty acids has a negative feedback effect on the glucagon secretion. Sympathetic stimulation consistently and parasympathetic stimulation under some conditions evoke glucagon release.

Mechanism of Action

Glucagon binds to the glucagon receptor, a G protein-coupled receptor, located in the plasma membrane of target cells and activates adenylate cyclase enzyme through stimulatory G-proteins. Adenylate cyclase synthesises cyclic adenosine monophosphate (cAMP), which activates protein kinase A (cAMP-dependent protein kinase). This enzyme, in turn, activates phosphorylase kinase, which, in turn, phosphorylates glycogen phosphorylase, converting into the active form called phosphorylase A. Phosphorylase A is the enzyme responsible for the release of glucose-1-phosphate from glycogen polymers.

Physiological Effects

Carbohydrate metabolism: The major metabolic effect of glucagon is on carbohydrate metabolism. It stimulates glycogenolysis and gluconeogenesis and decreases glycogen synthesis in the liver cells. During gluconeogenesis, amino acids from skeletal muscle and non-glucose compounds such as lactate and glycerol are converted to glucose. The net result is an increase in glucose concentration in the blood. Glucagon's action on carbohydrate metabolism is thus the opposite of insulin. Paradoxically, glucagon stimulates release of insulin.

Other effects: Glucagon at higher concentration produces lipolysis in adipose tissues and inhibits storage of triglycerides in the liver. This increases availability of fatty acids to energy systems of the body. In very high concentrations, it enhances force of contraction of heart, increases bile secretion and inhibits gastric secretion.

DISORDERS OF GLUCAGON

Deficiency of glucagon

Glucagon deficiency is not a common disorder in animals. Deficiency of glucagon results in hypoglycaemia with manifestations of GI disturbances such as nausea, vomiting, hunger, borborygmus (rumbling or gurgling sound produced by stomach and intestine) and abdominal discomfort. Deficiency of glucose in humans has been associated with neuroglycopenic manifestations such as headache, fatigue, weakness, stupor, ataxia and mood alterations.

Excess of glucagon

Abnormally-elevated levels of glucagon may be caused by glucagonoma, a rare tumour of the alpha cells of the pancreas. The elevated concentration of glucagon enhances blood glucose levels through the activation of gluconeogenesis and lipolysis. The net result is hyperglycaemia, decreased blood levels of amino acids (hypoaminoacidemia), anaemia, diarrhoea and weight loss. Diabetes mellitus also frequently results from the insulin and glucagon imbalance that occurs in glucagonoma.

TREATMENT OF GLUCAGON DISORDERS

I. Drugs Used in Deficiency of Glucagon

Glucagon

Glucagon is a polypeptide hormone produced by the α-cells of the pancreatic islets of Langerhans. Commercially, glucagon is extracted from bovine and porcine pancreas and is used occasionally in the treatment of acute hypoglycaemia.

Pharmacological effects

The mechanism of action and pharmacological effects of exogenous glucagon are essentially similar to those of endogenous hormone. After parenteral administration, glucagon produces rise in blood glucose levels. The hyperglycaemic action of glucagon is transient and may be inadequate if hepatic stores of glycogen are depleted.

Pharmacokinetics

Like other polypeptide hormones, glucagon is ineffective orally. After IV, IM or SC injection, glucagon produces effect within 10 minutes.

Side effects/Adverse effects

Side/Adverse effects reported in human beings include nausea, vomiting, diarrhoea and hyperglycaemia. Rarely hypersensitivity reactions may occur.

Contraindications and precautions

Glucagon use is contraindicated in insulinoma, phaeochromocytoma and glucagonoma. It should be cautiously administered to patients with a history of cardiovascular or renal disease. Glucagon must be reconstituted before administration and it must be used or refrigerated after reconstitution.

Drug interactions

Glucagon interacts with oral anticoagulants, increasing the tendency to bleed. Glucagon is incompatible in solution with most substances.

Clinical uses

Glucagon is used in both human and veterinary medicines in the treatment of acute hypoglycaemia induced by overdosage of insulin or oral antidiabetic drugs. Glucagon minimises the risk of neurological damage from hypoglycaemia. After the initial response to glucagon, patients should be given glucose or offered food to prevent recurrent hypoglycaemia. If glucagon therapy is not effective within 15 minutes, IV glucose should be administered.

In human medicine, glucagon has been tried in cardiogenic shock and respiratory heart failure with limited success. It is also used in the diagnosis of insulinoma and phaeochromocytoma. In phaeochromocytoma, it causes release of catecholamines from the tumour and may markedly increase blood pressure. If glucagon is used in phaeochromocytoma, the alpha-adrenoceptor blockers (e.g. phentolamine) should be at hand to counter excessive rise in blood pressure.

Dose

For acute hypoglycaemia

Dogs & cats : 0.02 – 0.03 mg/kg, SC, IM or IV. If no response occurs after 15 minutes, glucose should be administered by IV route (1 ml/kg of 50% glucose, IV infusion).

II. Drugs Used in Excess of Glucagon

Excess of glucagon secretion may be treated by both surgical and non-surgical methods. If the excess glucagon is due to glucagonoma, the only curative therapy is surgical removal of the tumour. This helps to reverse clinical signs in some patients. The non-surgical treatment of excess glucagon secretion includes administration of octreotide, a somatostatin analogue, which inhibits the release of glucagon. Doxorubicin and streptozotocin have also been used successfully to selectively damage alpha cells of the pancreatic islets.

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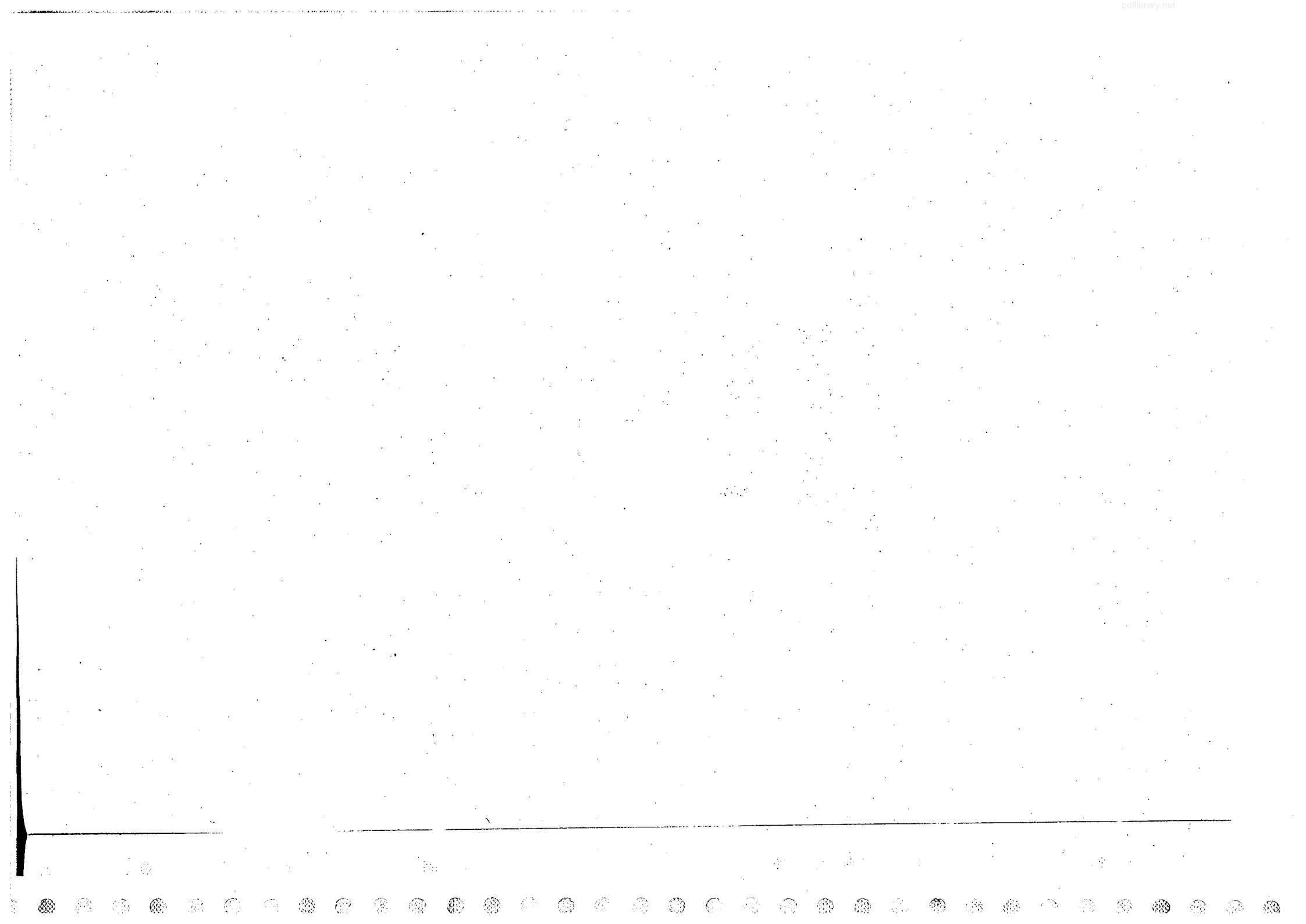
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SECTION-X
VITAMINS

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CHAPTER 54 FAT SOLUBLE VITAMINS

Classification
Fat soluble vitamins
  Vitamin A
    Absorption, transport and fate
    Mechanism of action
    Physiological functions
    Disorders of vitamin A
      Deficiency of vitamin A
      Excess of vitamin A
    Pharmacology of vitamin A
      Classification
      Synthetic preparations of vitamin A
        Structure and structure-activity relationship
        First generation retinoids
        Second generations retinoids
        Third generation retinoids
      Natural preparations of vitamin A
  Vitamin D
    Chemistry and source
    Types of vitamin D
      Absorption, transport, metabolism and excretion
      Mechanism of action
      Physiological functions
      Disorders of vitamin D
        Deficiency of vitamin D
        Excess of vitamin D
      Pharmacology of vitamin D
        Classification
        Vitamin D and metabolites
        Synthetic analogues of vitamin D and metabolites
        Miscellaneous agents
  Vitamin E
    Chemistry and source
    Absorption and fate
    Physiological functions
    Disorders of vitamin E
      Deficiency of vitamin E
      Excess of vitamin E
    Pharmacology of vitamin E
  Vitamin K
    Chemistry and source
    Absorption and fate
    Physiological functions
    Disorders of vitamin K
      Deficiency of vitamin K
      Excess of vitamin K
    Pharmacology of vitamin K
      Classification
    Natural vitamin K compounds
    Synthetic vitamin K compounds
      Menadione
      Derivatives of menadione

Vitamins are a group of poorly defined non-energy producing organic compounds which are needed in small quantities for normal body metabolism and lack of which causes deficiency diseases. The term 'vitamin' originated from 'vital amine' a name Casmir Funk in 1912 gave to thiamine (vitamin B_1). Subsequently the term was shortened as it was known that not all vitamins contain amine groups. With the exception of a few vitamins in some species, the vitamins cannot be synthesized in the body and must be supplied in adequate amounts in the diet. When lacking in diet, vitamins can cause specific metabolic deficits. In addition to dietary deficiencies, vitamin deficit can also occur due to their malabsorption, increased tissue need, inefficient utilisation, increased excretion, certain genetic abnormalities, and drug-vitamin interactions.

The pharmacological importance of vitamins is primarily in the prevention and treatment of specific deficiency diseases and when the diet in known to be vitamin deficient. They are also often used for general supportive therapy and during recovery from debilitating diseases. Many vitamins, often as multivitamins, are administered as 'tonic' with mineral or other dietary supplements. However, vitamins should not be overused or overprescribed because some of these agents, especially vitamin A and vitamin D, can be harmful as they accumulate in body and may cause pathological changes. Most of the vitamins can be synthesised commercially today.

CLASSIFICATION

Traditionally vitamins are categorised into two groups – fat-soluble vitamins and water soluble vitamins.

I. Fat soluble vitamins
   1. Vitamin A
   2. Vitamin D
      a. Vitamin D_2 (Ergocalciferol)
      b. Vitamin D_3 (Cholecalciferol)
   3. Vitamin E
   4. Vitamin K

II. Water soluble vitamins
   1. Vitamin B complex

a. Vitamin B_1 (Thiamine)
b. Vitamin B_2 (Riboflavin)
c. Vitamin B_3 (Nicotinic acid)
d. Vitamin B_5 (Pantothenic acid)
e. Vitamin B_6 (Pyridoxine)
f. Vitamin B_7 (Biotin)
g. Vitamin B_9 (Folic acid)
h. Vitamin B_{12} (Cyanocobalamin)
i. Choline
j. Inositol
k. Carnitine
l. Para-amino benzoic acid

2. Vitamin C (Ascorbic acid)

In recent times, vitamins are also classified by their biological and chemical activity. Thus, each vitamin now refers to a number of organic compounds, often called vitamer, all of which show the biological activity associated with a particular vitamin. Vitamers by definition are convertible to the active form of the vitamin in body and are sometimes inter-convertible to one another, as well.

FAT SOLUBLE VITAMINS

Fat-soluble vitamins are soluble in lipid solvents and comprise vitamins A, D, E and K. These vitamins are absorbed and transported by conventional lipid transport process and can be stored in body for long periods. In contrast to water-soluble vitamins, fat-soluble vitamins serve a broad range of functions such as enzymatic cofactors, signal transduction mediators and anti-oxidants and are liable to cause cumulative toxicity after regular ingestion of large amounts. Some vitamins interact with specific cellular receptors analogues to hormones. Lipid soluble vitamins usually have large hydrogen moieties in their chemical structures.

VITAMIN A

Originally the term vitamin A was applied to retinol, a monohydric alcohol, described as a growth promoting factor present in egg yolk, milk, butter and cod liver oil. Presently, the term vitamin A is used collectively for retinol and several other compounds possessing the biological activity of retinol. In nature, the vitamin A compounds are largely present as pure vitamin A compounds in animal tissues and as provitamin forms of vitamin A in plants. The pure vitamin A forms include two retinoids, the retinol (vitamin A_1) and the 3-dehydroxyretinol (vitamin A_2). The provitamins belong to the carotenoid family. One I.U. of vitamin A is equal to 0.3 µg of retinol; 5-8 µg beta-carotene has the same activity as 1 µg of retinol.

1. Pure Vitamin A/Retinoids: The retinoids refer to the chemical entity retinol or other closely related naturally occurring derivatives.

Vitamin A_1/Retinol: Vitamin A_1 or retinol, commonly referred to as vitamin A, is an isoprenoid compound containing a six-member carbocyclic ring and an eleven-carbon side chain. Retinol is present in esterified form (retinyl ester) in the mammalian tissues and marine fishes (mainly liver). A number of geometric isomers of retinol exist because of the possible cis-trans configurations around the double bonds in the same side chain; interconversion between isomers readily takes place in body. Fish liver oils contain mixtures of isomers. Although marine fish (e.g. cod, shark and halibut) liver oils are rich source of retinol, it is prepared nowadays synthetically. Egg yolk, butter and milk are good dietary sources of retinol.

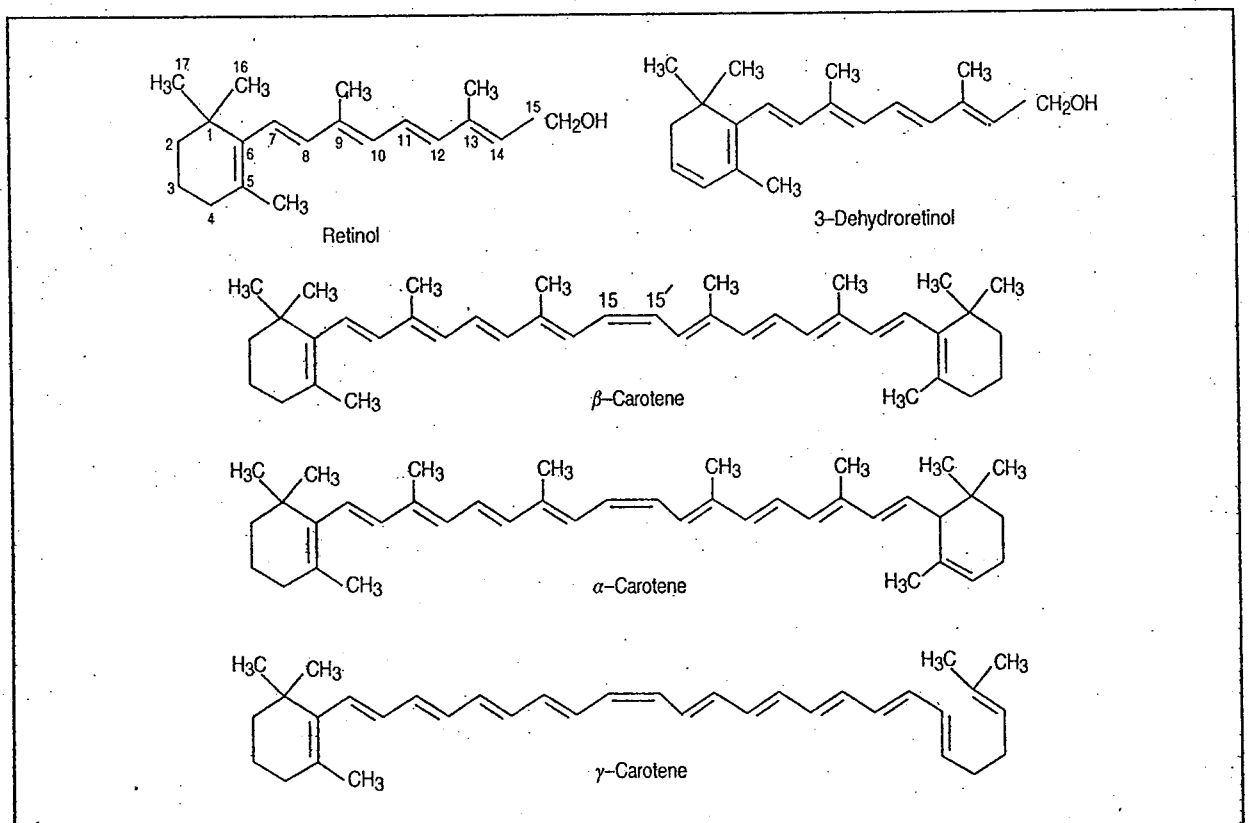


Fig. 54.1. Chemical structures of selected retinoids and carotenoids.

Vitamin A_2 /3-Dehydroxyretinol: Vitamin A_2 differs from vitamin A_1 only in having one additional double bond in its molecule (Figure 54.1). Vitamin A_2 is about 3 times less active than vitamin A_1 and is of little clinical significance. It is obtained mainly from fresh-water fish liver and occurs mostly mixed with retinol.

2. Proforms of vitamin A/Carotenoids: Carotenoids are reduced yellow biological pigments found mainly in green plant. In plants and prokaryotes, carotenoids occur in association with chloroplasts and serve as mediators of photo-energy related process by capturing energy from light. Carotenoids are rich in conjugated double bonds. In order to act as provitamin A, a carotenoid must contain an β-ionone structure. Although more than 600 carotenoids have been isolated, only about 50 appear to have some degree of vitamin A activity. Out of these, only four i.e. α-carotene, β-carotene, γ-carotene, and γ-cryptoxanthine are important. The carotenes and cryptoxanthine have no intrinsic vitamin A activity *per se*, but due to structural similarity to vitamin A, they are converted into vitamin A by enzymatic reactions in the liver and intestinal mucosa. The β-carotene, a symmetrical molecule (Figure 54.1), is cleaved in its centre to yield two molecules of retinol, while α- and γ-carotenes and γ-cryptoxanthine yield only one molecule of vitamin A. Cats, unlike other species, are unable to convert β-carotene to vitamin A and therefore require a dietary supply of pure vitamin A. Horses also do not covert carotene to vitamin A very easily.

Fresh green plants, good quality hay, carrots, tomatoes, egg yolk, milk, butter and cheese are important dietary sources of carotenoids. The richest source of carotenoids is red-palm oil, which contains 0.5 mg of mixed α- and β-carotenes per millilitre.

Absorption, Transport and Fate

Retinoids: Following ingestion, most of the retinyl esters in animal products are hydrolysed to retinol in the intestinal lumen by pancreatic enzymes and lipid hydrolases. Although they are lipophilic, the uptake of retinol by intestinal epithelial cells apparently occurs by a carrier-mediated process and is facilitated by presence of a cytosolic protein called cellular retinol binding protein (CRBP). Most of the retinol is re-esterified (mainly to palmitate) within the intestinal absorption cells into chylomicra (chylomicrons) which are released into the lymph. Appreciable quantities of retinol are absorbed directly into the circulation, where they are bound to the retinol-binding protein (RBP) in plasma. Most of the absorbed retinyl esters in chylomicra particle in lymph are carried to the liver, where about 75% of the retinol-derived products are stored as retinyl esters through a receptor mediated internalisation process. As the body needs vitamin A, retinyl ester in liver is hydrolysed and released as retinol bound to retinol-binding protein (RBP).

Excess retinol in liver cells is oxidised by the dehydrogenases in cytosol as well as by the microsomal oxidases associated with smooth endoplasmic reticulum. The microsomal enzyme system results in a variety of oxidation products as well as retinyl glucuronides. Some of these products are excreted into the intestine via bile. Several other water-soluble metabolites are also excreted in the urine and faeces.

Carotenoids: In contrast to retinol, about 40 to 60 percent (depending on the type) of dietary carotenoids are absorbed from the intestine. In the intestinal mucosal cells, some carotenoids are oxidised to retinals, which are then reduced to retinol and utilised (esterified and released into lymphatic). Some carotenoids are absorbed as such and circulate in association with lipoproteins. These circulating carotenoids are subsequently converted to retinol in numerous tissues including the liver.

Retinol bound to RBP circulates in body and reaches various target organs. The retinol-RBP complex binds to specific sites on the cell surface and then retinol is transferred to a membrane bound protein that appears to be closely related to the cellular retinol binding proteins (CRBP) present in intestinal absorption cells. This CRBP is present in virtually all tissues except cardiac and skeletal muscle, and ileal mucosa. In addition to its role in the uptake of retinol, CRBP functions as a reservoir of cellular retinol and delivers the vitamin to appropriate sites for its conversion to active compounds.

Mechanism of action

Vitamin A (Retinoids) mediates its action by binding to specific nuclear receptors called retinoid receptors. When bound to a retinoid, the retinoid receptors act as transcription factors, altering the expression of genes. This promotes genesis of specific mRNA and eventually synthesis of proteins. Two subtypes of retinoid receptors viz. retinoic acid receptors (RARs) and retinoid x receptors (RXRs) have been identified.

Physiological functions

Vitamin A has a number of important functions in the body. These functions are mainly related to vision, cell differentiation and tissue growth.

Vision: The mammalian retina contains two types of light sensitive photoreceptor cells, rod cells and cone cells. The rod cells are sensitive to light of low intensity, but not colours; therefore, they are involved in night vision. The cone cells act as receptors of high-intensity light and are responsible for colour visions. Vitamin A is more actively involved in the visual cycle in rod cells and its function is described below.

Visual cycle: Retinal rod cells contain a light sensitive pigment 'rhodopsin', which is synthesised by rods during dark adaptation. Rhodopsin (earlier called visual purple) is formed by the combination of protein opsin and 11-cis-retinal, the aldehyde of vitamin A. The 11-cis–retinal is initially formed from 11-cis-retinol in a reversible reaction. The 11-cis-retinal contains four carbon-carbon double bonds in its side chain out of which three are in trans form and the fourth at position 11,12 in cis-form (Figure 54.3). When a photon of light strikes rhodopsin, the bound 11-cis-retinal undergoes transformation and molecular changes, ending in the dissociation of the bleached rhodopsin to yield free opsin and all trans-retinal. The all trans-retinal undergoes isomerisation back to 11-cis-retinal. The 11-cis-retinal so formed now recombines with opsin to yield rhodopsin, thus completing the visual cycle (Figure 54.2). Alternatively, all trans-retinal can be reduced to all-trans retinol, which is then converted to 11-cis-retinol and then to rhodopsin.

The activated rhodopsin in the visual cycle interacts with another protein of the retinal rod called transducin (one of the proteins in transmembrane G-protein family). Transducin in turn stimulates a cyclic GMP-specific phosphodiesterase enzyme, which eventually causes a decrease in cGMP. This sequence of events leads to a decreased conductance of cGMP-gated Na^+ channels and alterations in membrane polarisation, which activate optic nerve signal propagation (Figure 54.2).

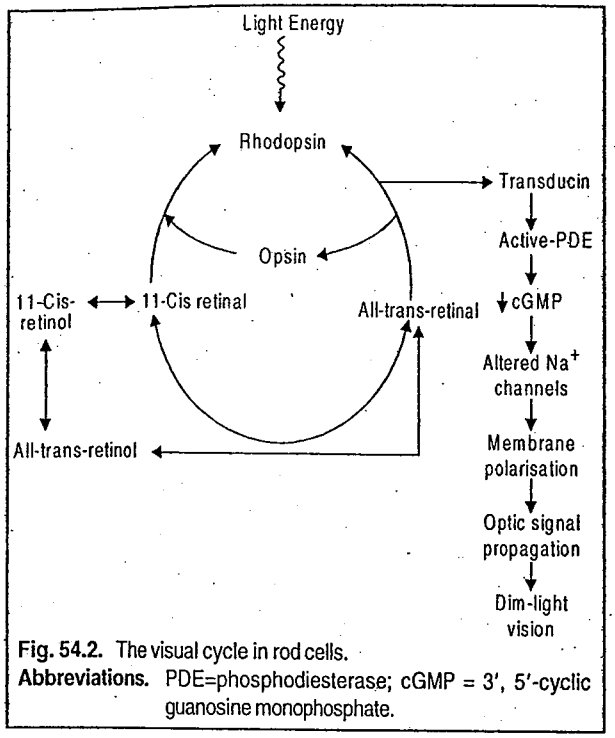


Fig. 54.2. The visual cycle in rod cells.
Abbreviations. PDE=phosphodiesterase; cGMP = 3', 5'-cyclic guanosine monophosphate.

In dietary vitamin A deficiency, formation of rhodopsin and its subsequent complexes are interfered with. This decreases the sensitivity of the visual apparatus so that light of low intensity in not perceived. This leads to clinical condition of nyctalopia or night blindness. Irreversible structural changes with permanent night blindness occur if the deprivation is long-term. In vitamin A deficiency, cones are also affected in a similar manner, but effect is less in comparison with rods. In cones, a similar pigment iodopsin is synthesised that is responsible for vision in bright light, colour vision and primary dark adaptation.

Epithelial cells: Vitamin A maintains normal structure and function of epithelial cells all over the body. It promotes mucus secretion and inhibits keratinisation of the epithelial cells. In this process it appears that within the target cells, all-trans-retinol is oxidised and isomerised to 13-cis retinoic acid, which subsequently binds to retinoic acid specific cytosolic receptors which are members of the thyroid/steroid receptors. These retinoic acid receptors then control the expression of various proteins important to mucus formation and cytoskeletal integrity (e.g. fibronectin) and reduce the synthesis of other (e.g. collagenase and certain species of keratin). Retinoic acid shares some, but not all actions of retinol. It is not essential for vision or reproductive functions, but is very potent in promoting growth and controlling differentiation of maintenance of epithelial cells.

Vitamin A can influence also the expression of receptors for certain hormones and growth factors and thus can influence the growth, differentiation and function of target cells indirectly. With vitamin A deficiency, squamous, keratin-enriched cells replace mucus-secreting cells. The lining of the GI tract is quite sensitive to presence or absence of vitamin A.

Immunity: Vitamin A appears to be required for proper antibody response, normal lymphocytic proliferations and killer cell function. Vitamin A deficiency is associated with increased susceptibility to bacterial, parasitic and viral infections. In vitamin A deficiency, the damaged epithelial cells often become infected. Therefore vitamin A has been called an 'anti-infection' vitamin.

Reproduction: Vitamin A is needed for the maintenance of spermatogenesis and foetal development. Deficiency of vitamin A may result in failure of reproduction associated especially with atrophy of germinal epithelium of the testes.

Other functions: Vitamin A is considered important for growth and cell differentiation. It has got important role in carbohydrate, fat and protein metabolism. Vitamin A is reported to possess anticarcinogenic effect because its deficiency has been associated with increased susceptibility to carcinogenesis.

Disorders of Vitamin A

Deficiency of Vitamin A / Avitaminosis A

Deficiency of vitamin A (Avitaminosis A) is manifested in all species but is commonest in growing cattle fed on poor quality hay. Since vitamin A is stored in liver, deficiency symptoms appear only after long-term deprivation. Deficiency of vitamin A in animals may occur due to insufficient vitamin in diet (e.g. poor quality hay where much of vitamin A is lost due to storage), prolonged administration of liquid paraffin as laxative and various pathological conditions (e.g. pancreatic insufficiency, liver disease and kidney disease). Factors which impair lipid absorption and transport also influence vitamin A status.

The appearance, signs and symptoms of vitamin A deficiency in a number of species have been described. Important manifestations of avitaminosis A in different species include night blindness, keratomalacia (softening and drying of cornea), corneal keratinisation, excessive lachrymation and hyperkeratosis of skin. Nervous symptoms, decreased growth rates, sterility, abortions, foetal malformations, rough hair coat with scaly, cracked skin and dry mucous membranes are also commonly seen. Although the requirement of vitamin A for a given animal depends on age, sex, rate of growth and reproductive status, the daily requirement in general ranges from 100 to 200 I.U./kg body wt. In animals, 1000 to 8000 I.U/kg of feed of vitamin A is considered adequate requirement for most species.

Excess of Vitamin A / Hypervitaminosis A

Regular ingestion of excess of vitamin A in diet or vitamin A containing supplements leads to hypervitaminosis A. It may be characterised by anorexia, irritability, lethargy, dry skin, pruritus with desquamation, increased intracranial tension and chronic liver disease. Overdosage from excessive dietary intake of liver most commonly occurs in dogs and cats and may result in vertebral fusion. In foals, excessive intake of vitamin A may result in a decreased growth rate with increased risk of developmental orthopaedic disease. Excess vitamin A is also teratogenic in animals and man. Carotenoids, unlike retinoids, are generally non-toxic.

PHARMACOLOGY OF VITAMIN A

Vitamin A is a generic name used for compounds possessing the biological activity of retinol. Pharmacologically, the term includes retinol, other closely related forms of vitamin A and various synthetic preparations which exhibit vitamin A activity. All synthetic vitamin A compounds are structurally related to retinol, therefore they are commonly referred to as retinoids. Vitamin A is used clinically mainly to treat skin disorders (acne and psoriasis) and certain forms of cancer.

Classification

I. Synthetic preparations of vitamin A/Synthetic retinoids
   1. First generation retinoids
      e.g. retinol, tretinoin, isotretinoin and alitretinoin.

2. Second generation retinoids
   e.g. etretinate and acitretin.
3. Third generation retinoids
   e.g. bexarotene, tazarotene and adapalene.

II. Natural preparations
   e.g. cod liver oil and halibut liver oil.

I. Synthetic Preparations of Vitamin A/Synthetic Retinoids

Synthetic retinoids are used widely in human medicine, but their use in clinical veterinary medicine has not been well established. Occasionally, these are used in dogs for the treatment of keratinisation disorders, particularly primary seborrhoea. Synthetic retinoids are in general more toxic than natural preparations of vitamin A. Teratogenicity may occur with all synthetic retinoids; therefore they are contraindicated in pregnancy.

Structure and structure-activity relationship

The basic structure of the retinoid molecule consists of a cyclic group, a polyene side chain and a polar end group (Figure 54.3). Many different geometric isomers of retinol, retinal and retinoic acid are possible as a result of either a *trans* or *cis* configuration of four of the five double bonds found in the polyene side chain. The conjugated system formed by alternating C=C double bonds in the polyene side chain are responsible for the colour of retinoids (typically yellow, orange or red). Hence, many retinoids are chromophores. Alternation of side chains and end groups creates the various classes of retinoids. First and second generation retinoids are more flexible due to the alternating single and double bonds; therefore they are capable of interacting with several retinoid receptors. Third generation retinoids interact with fewer retinoid receptors due their comparatively less flexible structure than the first and second generation retinoids.

1. First Generation Retinoids

Retinol

Retinol is one of the natural vitamin A present mainly in the animal tissues. Commercially, it is obtained mainly from synthetic sources and is used as a palmitate or acetate salt. Unlike naturally occurring retinol, synthetic retinol is the all-trans isomer. The pharmacological effects of synthetic retinol are essentially similar to the biological effects of retinol. Various preparations of synthetic vitamin A for oral and parenteral administration are available for use in human and veterinary medicines.

Dose

Dogs & cats : 600 – 800 I.U./kg, PO, 2 times daily.

Tretinoin

Tretinoin is all-trans retinoic acid (all trans vitamin A acid) prepared synthetically. As the all trans-retinoic acid is the active form of vitamin A in all tissues except retina, the tretinoin possesses significant therapeutic value and is available as a drug. It increases dermal and granular layer thickness and decreases melanocyte activity. It is a potent comedolytic agent i.e. it promotes lysis of keratinocytes hence formation of comedones (a plug of sebaceous and dead skin material stuck in the opening of a hair follicle). In humans, tretinoin decreases wrinkling and prevents rough and leathery skin formation; pigmented spots also tend to fade

Tretinoin is used mainly in human medicine as 0.01 – 0.1 percent topical preparation for the treatment of dermatoses. It is found to be highly efficacious in acne (see also Chapter 73). Topical tretinoin (0.025% cream) may be efficacious for the treatment of feline acne, but it should be used very sparingly to avoid serious tissue irritation. Important side/adverse effects of tretinoin in humans include erythema, peeling, burning and stinging. Oral administration of tretinoin for the treatment of dermatoses is not practiced because it may produce severe symptoms of hypervitaminosis A. Teratogenic risk with topical retinoic acid is little because of low blood level produced.

Dose

For feline acne

Cats : 0.025% cream, topically, once daily.

Isotretinoin

Isotretinoin is the 13-cis-retinoic acid prepared synthetically. Isotretinoin is nearly as potent as tretinoin in many of its actions on epithelia tissue but is less potent in producing the toxic symptoms of hypervitaminosis A. Isotretinoin normalises keratinisation of the follicular epithelium and reduces sebum synthesis. In humans, it also reduces *Proprionobacterium acnes*, the organism that produces inflammation in acne. Isotretinoin and tretinoin are interconverted *in vivo* to some extent (20-30%).

Isotretinoin is administered orally because it is not effective topically. It has a high level of lipophilicity and is best absorbed when taken after a high-fat meal. Isotretinoin is primarily (99.9%) bound to plasma proteins, mostly albumin. It is metabolised and excreted mainly in urine and faeces. The half-life of isotretinoin in human beings varies between 6 and 36 hours. Adverse effects of isotretinoin are dose-dependent and include mainly skin and mucous membrane manifestations like epistaxis, dry eyes, blepharoconjunctivitis, dry mucous membranes and erythematous eruptions. Systemic manifestations include myalgia, arthralgia and increased liver enzymes, which can be minimised with short-term use the drug. Cats may have high incidence of side effects. Pregnancy is absolute contraindication to use of isotretinoin due to potential teratogenicity.

Isotretinoin is used mainly in human medicine for the treatment of severe skin conditions such as cystic acne vulgaris. The veterinary applications of isotretinoin include selected abnormalities of sebum production such as primary idiopathic seborrhoea and comedo syndrome.

Dose

Dogs (Loading): 1 – 3 mg/kg, PO, 1 to 2 times daily. Maximum dose should not increase 3 - 4 mg/kg/day

(Maintenance) : 0.5 – 1 mg/kg, PO, every alternate day.

Alitretinoin

Alitretinoin is 9-cis-retinoic acid. It is primarily approved as an antineoplastic agent for the treatment of skin lesions in AIDS-related Kaposi's sarcoma.

2. Second Generations Retinoids

Etretinate

Etretinate is a synthetic aromatic retinoid and an ethyl ester of the active compound acitretin. Etretinate is most effective for the inflammatory types of psoriasis, including pustular and erythrodermic diseases. It normalises keratin expression in epidermal cells, suppresses chemotaxis, decreases stratum corneum cohesiveness and may impairs cytokine function. Its therapeutic effect is probably due to a combination of these factors.

Etretinate is administered orally with a bioavailability of about 50% in human beings. In body, it is converted to an active metabolite acitretin. Etretinate is highly lipid soluble hence its plasma half-life depends on the duration of treatment. The half-life of etretinate after single dose in humans varies from 7 to 9 hours. However, after continuous administration for 1 year, the half-life gradually increases to about 60 to 170 days due to its storage and release from adipose tissue. It is detectable in the plasma for up to three years following therapy.

The adverse effects of etretinate are similar to those of isotretinoin. However the conjunctival symptoms are less common, while the hair loss, cutaneous exfoliation bruising, and liver dysfunction are more common with etretinate. Etretinate is a teratogen and may cause birth defects long after use. Its teratogenicity precludes use in pregnant females. Etretinate has largely been replaced by acitretin, a safer metabolite of etretinate.

Dose

Dogs : 0.75 – 1 mg/kg, PO, once daily.

Cats : 1 – 2 mg/kg, PO, once daily.

Acitretin

Acitretin is the major acid metabolite of etretinate that has been prepared synthetically as a second generation aromatic retinoid. Its pharmacological activity is similar to that of etretinate except that it is not lipid soluble and hence does not accumulate in any tissue on continuous exposure. However, some portion of acitretin is esterified to produce etretinate *in vivo*, which eliminates the advantage of short half-life (humans 2 days) of acitretin. It is approved mainly for treatment of psoriasis in human medicine. Acitretin has almost replaced the older etretinate.

3. Third Generation Retinoids

The third generation retinoids contain two aromatic rings which serve to restrict flexibility of the polyenoic side chain. The third generation aromatic retinoids are highly potent and are sometimes called arotinoids. Various arotinoids are in the developmental stages.

Bexarotene

Bexarotene is a third generation retinoid and an oral antineoplastic agent. It binds to retinoid X receptors (RXR), as

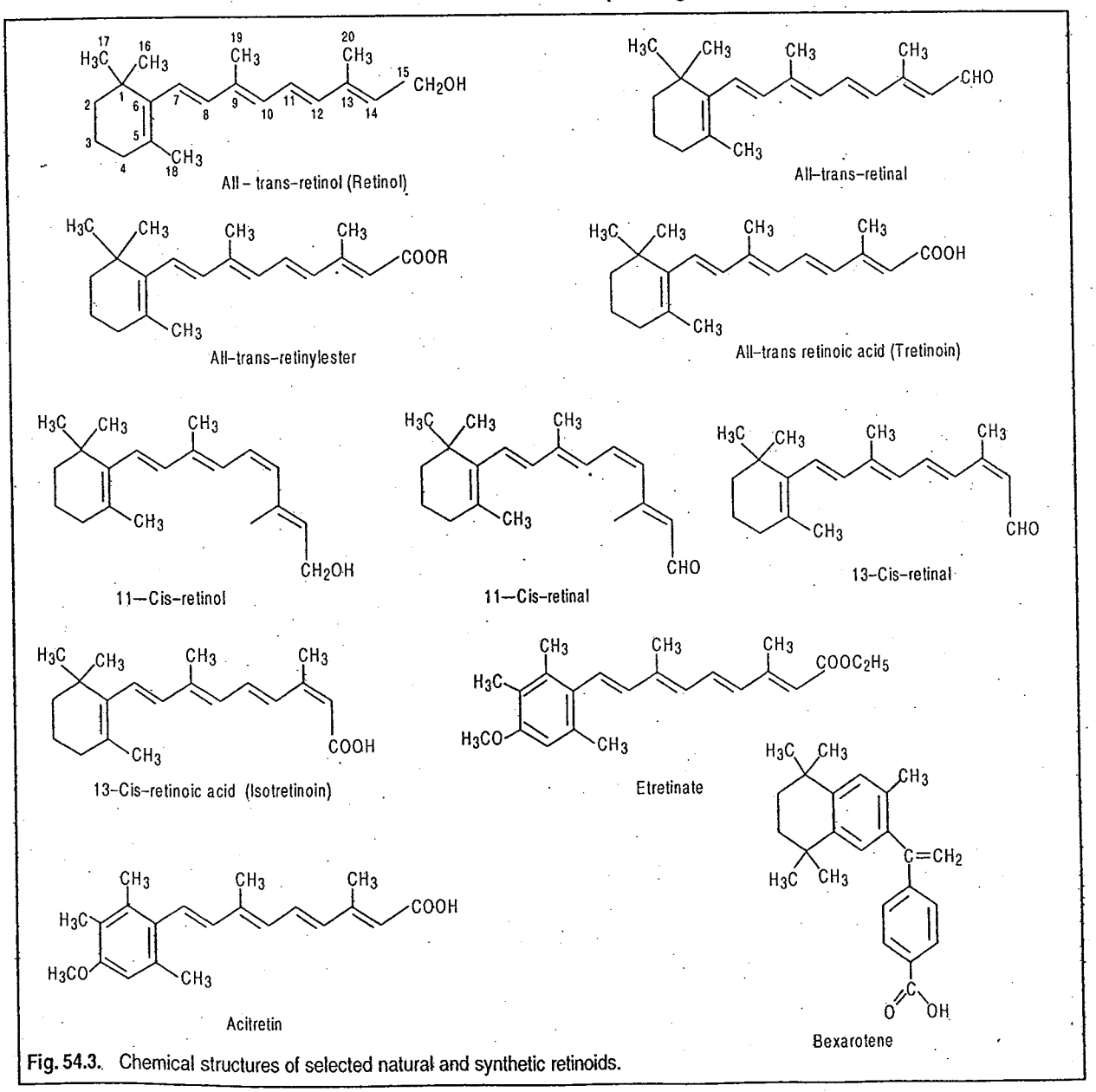


Fig. 54.3. Chemical structures of selected natural and synthetic retinoids.

opposed to the retinoic acid receptors (RAR). The RXRs located primarily in visceral organs such as the liver and kidney regulate the expression of genes which control cellular differentiation and proliferation. Bexarotene is primary indicated for treatment of cutaneous T cell lymphoma in human medicine. It may also be used in some other types of cancers including lung cancer, breast cancer and Kaposi's sarcoma.

Other drugs

Tazarotene and adapalene are other third generation retinoids approved largely for topical use in human patients. Tazarotene is used for treatment of psoriasis, acne and sun damaged skin. Adapalene is recommended for the treatment of mild-moderate acne and also keratosis pilaris and some other skin conditions.

II. Natural Preparations of Vitamin A

Natural preparations of vitamin A include cod-liver oil, halibut liver oil and concentrated solutions of vitamin A. Unlike synthetic retinoids, natural preparations contain mixtures of retinol stereo-isomers. These are commonly used in veterinary medicine.

Cod-liver oil

Cod liver oil is a clear, yellow, oil liquid with a fishy odour and taste. Cod-liver oil is a fixed oil extracted from the fresh livers of *Gadus morrhuae* (commonly called cod fish) and other species of the family *Gadidae*. Cod liver oil has high levels of the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and very high levels of vitamin A and vitamin D. It contains in each gram at least 600 BP I.U. (850 USP I.U.) of vitamin A and at least 85 I.U. of vitamin D. It is mainly used a supplementary source of vitamin A and D in both human and veterinary medicines. Cod liver oil is used as eye drops and also as a vehicle for skin therapeutics.

Dose

Dogs & cats : 0.5 – 8 ml (total), PO, once daily.
Sheep & pigs : 4 – 15 ml (total), PO.
Cattle & horses: 15 – 60 ml (total), PO.

Halibut liver oil

Halibut liver oil is a fixed oil obtained from the fresh or suitably preserved livers of halibut species of the genus *Hippoglossus* (Family Pleuronectidae). It is similar in character to cod liver oil but its vitamin content is very high. It contains in each gram not less than 30,000 BP I.U. of vitamin A and about 3000 BP I.U. of vitamin D. Halibut liver oil is a supplementary source of vitamins A and D, but is more expensive and rarely used other than in capsules for small animal treatment.

Dose

Dogs & cats : 0.075 – 0.3 ml (total), PO, daily.
Horses, cattle, sheep & pigs : 0.2 – 0.3 ml/kg, PO, daily.

Concentrated solutions of vitamin A

It is fortified fish liver oil or a solution of vitamin A in a bland oil (e.g. arachis oil). It contains between 45,000 and 55,000 units of vitamin A per gram.

Dose

Large animals : 100 – 200 I.U./kg, PO, daily.

VITAMIN D

Vitamin D is the generic name applied to a group of fat-soluble seco-steroids those exhibit the biological activity of either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3), the two major physiologically relevant forms of antirachitic agents (agent that has ability to prevent or cure rickets). Vitamin D compounds promote the proper utilisation of calcium and phosphorus, thereby producing growth in younger animal together with proper bone and tooth formation. Each I.U. of vitamin D is equivalent to the antirachitic activity of 0.025 µg of crystalline vitamin D_3. The requirements for poultry are expressed in International Chick Unit (I.C.U).

Chemistry and source

The vitamin D compounds are a family of 9,10-secosteroids derived from several plant and animal sterols. Secosteroids are very similar in structure to steroids except that two of the B-ring carbon atoms (C9 and C10) of the typical four steroid rings are not joined. Two such vitamin compounds viz. ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) are important in human and veterinary therapeutics. These two forms of vitamin D are produced in a two-step reaction when their respective sterols absorb ultraviolet radiation and undergo photolysis, followed by thermal isomerisation (Figure 54.4). The structural difference between vitamin D_2 and vitamin D_3 is in their side chains. The side chain of D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24. Both vitamins D_2 and D_3 require further activation in body to become biologically active.

Types of Vitamin D

Several types of vitamin D exist in nature. Among these, vitamins D_2 and D_3 are clinically important.

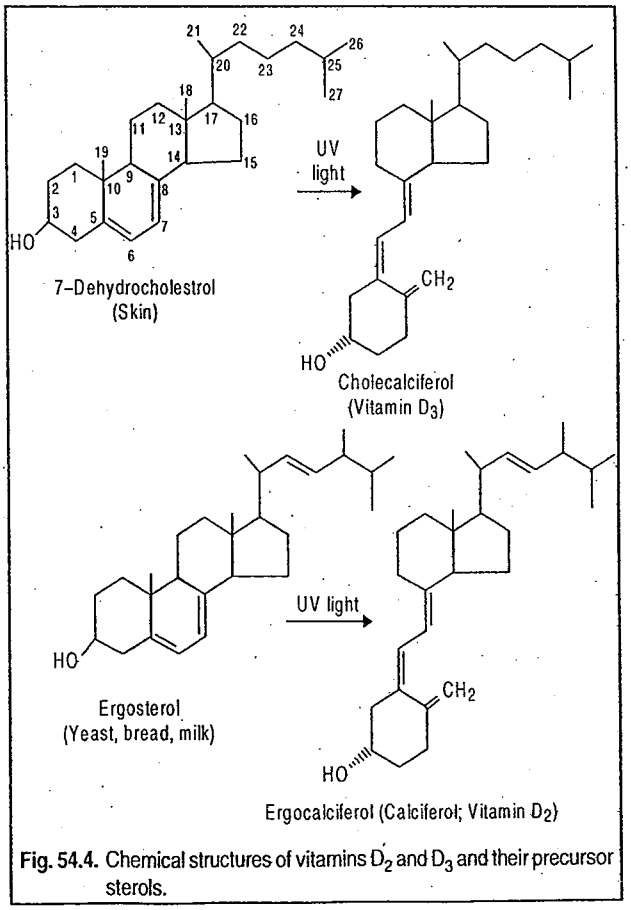


Fig. 54.4. Chemical structures of vitamins D_2 and D_3 and their precursor sterols.

Ergocalciferol /Vitamin D_2: Ergocalciferol (Calciferol) or vitamin D_2 is produced by UV irradiation of ergosterol, the provitamin for vitamin D_2. Ergosterol is mainly present in yeasts and fungi up to 2 percent concentration of the dried substance. Each milligram of ergocalciferol contains 40,000 units of antirachitic activity. Vitamin D_2 is the active constituent in a number of commercial preparations as well as in the irradiated bread and irradiated milk.

Cholecalciferol / Vitamin D_3: Cholecalciferol is synthesised from 7-dehydrocholesterol, the principal provitamin found in animal skin on exposure to sunlight. Although in most animals, 7-dehydrochlesterol is abundant in skin, the skin of dogs and cats contains only small quantities of this provitamin D_3. Therefore, these animals are solely dependent on the diet of vitamin D. The vitamin D_3 also occurs naturally in fish-liver oils along with vitamin A. Vitamin D_3 is used as reference standard for assays of antirachitic compounds. Vitamin D_3 is equally potent for mammalian and avian species, whilst vitamin D_2 is equally active as vitamin D_3 for mammalian species, but has only about one fiftieth of the activity for avian species.

Vitamin D_1: Vitamin D_1 is a lumisterol-ergocalciferol mixture found in food and originally mistaken to pure vitamin D.

Vitamin D_4: Vitamin D_4 is the activated 22,23-dihydroergosterol. It is less effective as an antirachitic than the vitamins D_3 and D_2.

Vitamin D_5: Vitamin D_5 (Sitocalciferol) is irradiated 7-dehydrositosterol. Vitamins D_5 and D_4 occur naturally in the oils of some fish.

Absorption, Transport, Metabolism and Excretion

Absorption and transport: Vitamin D_2 is absorbed from the small intestine. Bile is essential for adequate absorption of vitamin D. After absorption (vitamin D_2) or release from skin (vitamin D_3), vitamin D circulates in blood in association with vitamin D binding protein, a specific α-globulin. Both dietary and intrinsically synthesised vitamin D requires activation to become biologically active. They follow the same metabolic pathways to yield the final functional forms (Figure 54.5).

Metabolism and excretion:

Vitamin D_3: Vitamin D_3 has a biological half-life of about 24 hours. In the initial step, it is converted to 25-hydroxy-cholecalciferol (25-hydroxy vitamin D_3; 25-OH D_3) or calcifediol in the liver. The 25-OH D_3 enters the circulation and is carried by vitamin D-binding globulin. It has half-life of about 3 weeks and is the major form of vitamin D_3 in the body. In kidneys, the 25-OH D_3 is converted to 1,25-dihydroxycholecalciferol (1,25-dihydroxy vitamin D_3; 1,25-$(OH)_2$ D_3) or calcitriol (Figure 54.5), the biologically active form of vitamin D. The calcitriol is believed to be about 10 times more potent than cholecalciferol, but has half-life of only about 4-6 hours. This step of metabolic conversion is tightly regulated by a specific renal tubular 1α-hydroxylase enzyme. This enzyme is activated or induced by dietary deficiency of vitamin D, calcium and phosphate. It is also stimulated by parathormone and probably oestrogen and prolactin. Conversely, its activity is suppressed by high calcium, phosphate and vitamin D intake; calcitriol also inhibits it in a feedback manner. Under normocalcaemia or hypocalcaemia and in the absence of parathormone, the kidneys instead convert 25-OH D_3 to the less active form 24,25-dihydroxycholecalciferol (24,25-dihydroxy vitamin D_3; 24,25-$(OH)_2$ D_3). In addition to 1,25-$(OH)_2$ D_3 and 24,25-$(OH)_2$ D_3, more than 20 other hydroxylated intermediates and end products have been identified; functional role for these products have not been clearly defined.

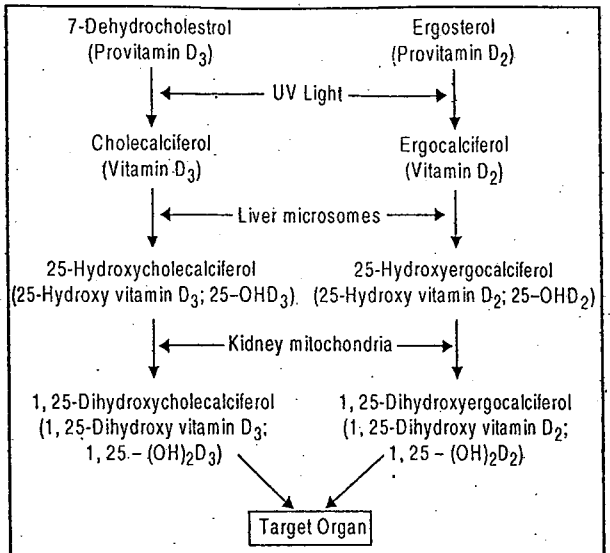


Fig. 54.5. Photobiogenesis and metabolic pathways for vitamin D production and metabolism.

Vitamin D_2: The biotransformation of ergocalciferol (D_2) follows the same metabolic pathway as the vitamin D_3. It is first converted to 25-hydroxyergocalciferol (25-OH D_2) in the liver and then to 1,25-hydroxyergocalciferol (1,25-$(OH)_2$ D_2) in the kidney. The 1,25-$(OH)_2$ D_2 is the active form of vitamin D_2.

Mechanism of Action

The mechanism of action of vitamin D broadly resembles that of steroid and thyroid hormones. In this process, 1,25-$(OH)_2$ D [1,25-$(OH)_2$ D_2 and 1,25-$(OH)_2$ D_3], the active form of vitamin D binds to the specific vitamin D receptor (VDR), which is principally located in the cytoplasm of target cells. When vitamin D binds to the receptor, the ligand-receptor complex translocates to the cell nucleus, where it acts as a transcription factor that modulates the gene expression to regulate the synthesis of specific proteins (i.e. binding to vitamin D receptor in cytoplasm → translocation of ligand-receptor complex to nucleus → interaction of complex with DNA → synthesis of m-RNA → regulation of protein synthesis). Receptors of 1,25 $(OH)_2$ D are distributed widely throughout the body and belong to the superfamily of steroid and thyroid hormones. Vitamin D receptor activation in the intestine, bone, kidney and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood (with the assistance of parathyroid hormone and calcitonin) and to the maintenance of bone content. Some for the action of vitamin D occur quickly (within minutes) and, therefore, appears to be exerted by mechanisms not involving gene regulation.

Physiological Functions

Vitamin D is involved mainly in the regulation of calcium homeostasis. The vitamin in a manner parallel to that of calcium also affects phosphate metabolism.

Calcium homeostasis: Vitamin D maintains the normal concentration of Ca^{++} and phosphate in plasma by facilitating their absorption from small intestine, by interacting with parathormone to enhance their mobilisation from bone, and by decreasing their excretion by kidneys.

Intestinal absorption of calcium: The 1,25-$(OH)_2$ D, the active form of vitamin D, enhances the intestinal absorption of Ca^{++} and phosphate by increasing the synthesis of a carrier protein for Ca^{++} called 'calcium binding protein or calbindin'. The active form of vitamin D also stimulates the production of calcium- and sodium-dependent ATPases, which reside on the basolateral membrane surface of the intestinal cell, thus facilitating movement of calcium out of the cells into circulation. It has been suggested that vitamin D receptor promotes endocytotic capture of calcium and its transport across duodenal mucosal cells in vesicular form.

Effects on bone: Vitamin D plays important role in both bone resorption and bone deposition. Vitamin D in small quantities promotes bone calcification and is required for normal mineralisation during skeletal growth and remodelling of bone. Although osteoblastic cells express vitamin D receptors and respond to it, the 1,25-$(OH)_2$ D appears to help bone mineralisation indirectly by enhancing Ca^{++} and phosphate absorption from the intestine. In contrast, physiological and extreme quantities of vitamin D promote mobilisation of Ca^{++} from bone; large doses cause excessive turnover. The mechanism of this action of vitamin D is not clear, but is believed to result from the effect of 1,25$(OH)_2$ D that promotes recruitment of differentiation of osteoclast precursor cells to the resorption sites. This action of vitamin D on calcium and phosphate resorption from bone is independent of but facilitated by parathormone.

Effects on kidneys: Vitamin D enhances proximal tubular reabsorption of both calcium and phosphate in kidneys, but the action is less marked than the parathormone. However in hypervitaminosis, excess calcium is excreted in the urine.

Other effects: Vitamin D receptors have been found in a large number on various cell-types where they perform functions not related to calcium homeostasis. In pancreatic β-cells, 1,25$(OH)_2$ D has been found important for normal insulin secretion. Vitamin D affects maturation and differentiation of mononuclear cells and influence cytokine production. Vitamin D also inhibits epidermal proliferation and promotes epidermal differentiation. Its effects on neuronal and skeletal muscle function have also been demonstrated.

In recent times, some scientists have suggested that vitamin D should be considered as a hormone and not a vitamin because:

a. It is synthesised in the skin and under ideal conditions may not be required in the diet.
b. It is transported in blood, activated and then acts on specific receptors in target tissues.
c. Feedback regulation of vitamin D occurs by Ca^{++} and also by itself.

Disorders of Vitamin D

Deficiency of Vitamin D

Deficiency of vitamin D is generally caused by insufficient solar irradiation of animals or their feed. Deficiency of vitamin D results in inadequate absorption of calcium and phosphate resulting in failure of bone to calcify correctly. Further, decrease in plasma calcium stimulates parathormone secretion, which acts to restore plasma Ca^{++} at the expense of bone. These factors lead to rickets in young animals and osteomalacia in adults.

Rickets is characterised by softening of the bones with associated skeletal deformities. The lack of ossification leads to enlarge painful joints, bending on long bones, lameness and general tenderness of body when touched. Osteomalacia is the equivalent disease to rickets in adults and is characterised by generalised accumulation of unmineralised bone matrix associated with bone pain and tenderness. The weakened bones often break or become deformed. In the less severe or early stages, a decrease in appetite and efficiency of food utilisation cause poor weight gains in growing animals and poor productivity in adults.

Nutritional requirement of vitamin D in animals vary widely with age, breed, climate, pregnancy and lactation. For examples, fast growing breed of animals require more of vitamin than the slow growing breeds; pregnant and lactating animals require more than non-pregnant animals; animals in arctic or subarctic climates require more than those in temperate or equatorial areas; and greater quantities of vitamin D are required in winter than in summer. Further, the requirement of vitamin D depends also on the calcium and phosphorus ratio in diet. Under optimal conditions (calcium and phosphorus ratio of 2:1), requirement of vitamin D is minimal, but as the ratio changes vitamin D requirements increase. In general, the daily requirement of vitamin D in dogs is 10-20 I.U./kg body wt. and in large animals is 6-10 I.U./kg body wt.

Excess of Vitamin D

Excess of vitamin D in diet can cause hypercalcaemia and calcification of soft tissue, particularly in the blood vessels of lungs, kidneys and heart. Toxicity tends to be chronic in nature since vitamin D is slowly metabolised. Very high concentration of vitamin D (about 100 times the requirements) can eventually lead to a negative calcium balance due to enhanced bone resorption. Hypercalcaemia and hypervitaminosis D is due solely to very high circulating levels of 25-OH D; plasma concentration of calcitriol and parathormone are both suppressed. Some plants such as *Solanum* spp., *Cestrum diurnun*, and *Trisetum flavescens* contain compounds with vitamin D activity; therefore consumption of these plants by grazing animals cause induced vitamin D toxicity and calcinosis. Rodenticides containing cholecalciferol as active ingredient may result in toxicity, if consumed by companion animals.

Pharmacology of Vitamin D

Vitamin D therapy is necessary for absorption of orally administered calcium and normalisation of calcium therapy. Initially, the term vitamin D was used only for ergocalciferol and cholecalciferol (vitamin D_3), but presently the term designates a range of natural and synthetic compounds exhibiting the biological activity of ergocalciferol (or cholecalciferol). These vitamin D compounds vary in potency, time of onset (time to steady state concentrations) and cost.

Classification

I. Vitamin D and metabolites
   e.g. cholecalciferol, ergocalciferol, calcitriol and calcifediol.
II. Synthetic analogues of vitamin D and metabolites
   e.g. alfacalcidol, dihydrotachysterol, paricalcitol, calcipotriol and tacalcitol.
III. Miscellaneous agents
   e.g. cod liver oil and halibut liver oil.

I. Vitamin D and Metabolites

Cholecalciferol

Cholecalciferol (Vitamin D_3, colecalciferol, calciol, activated 7-dehydrocholesterol) is a seeosteroid that occurs naturally in

cod-liver and other fish-liver oils. Cholecalciferol is produced commercially by the irradiation of 7-dehydrocholesterol extracted from lanolin found in sheep's wool. As a drug it is available mainly as granules for oral ingestion and oily solution for intramuscular injection. One mg of cholecalciferol contains about 40,000 I.U. of vitamin D. The pharmacological effects and biotransformation of cholecalciferol are essentially similar to those of endogenous hormone.

Cholecalciferol is well absorbed from intestine in the presence of bile salts and appears to have good bioavailability. It is stored in body, mostly in adipose tissues for many months. Steady state equilibrium between tissues and blood takes several weeks to achieve and normocalcaemia may not occur for up to 4 weeks after therapy has begun. Excessive dosage or prolonged use of vitamin D may result in hypercalcaemia, calcification of blood vessels and organs, and excess bone loss. Early symptoms of hypercalcaemia may include nausea, vomiting and anorexia, followed by polyuria, polydipsia, weakness, fatigue, somnolence, dry mouth, vertigo, tinnitus and ataxia. Kidney function may become impaired and metastatic calcifications (calcium deposition in organs throughout the body) may occur, particularly affecting the kidneys. Treatment involves stopping the intake of vitamin D or calcium and lowering the calcium levels with frequent monitoring of calcium levels. Acidification of urine and corticosteroids may be necessary.

Cholecalciferol is inexpensive and widely available. Therapeutically, vitamin D_3 is used mainly in the prevention of hypocalcaemia (e.g. milk fever). It is also used in the prevention and treatment of hypopanthyroidism, rickets and osteomalacia. Weekly followed by monthly serum calcium rechecks for 6 months should be done to monitor calcium levels. The 25-hydroxy vitamin D (calcifediol) blood test is also used to determine the level of vitamin D in the body.

Dose

Dog : 500 – 2000 I.U./kg/day, PO.

Cattle : 250 mg (total), IM, 2 to 8 days before calving.

Ergocalciferol/Vitamin D_2

Ergocalciferol (Calciferol) or vitamin D_2 is a white crystalline, odourless and tasteless powder that is available commercially for both oral and intramuscular administration. The pharmacology of ergocalciferol is similar to cholecalciferol, but it appears to have lower bioavailability due to lesser absorption than the vitamin D_3.

Dose

Dogs & cats : 500 – 2000 I.U./kg/day, PO.

Calcitriol

Calcitriol (1,25-dihydrocholecalciferol, 1,25-$(OH)_2$ D_3) is the hormonally active form of vitamin D. It is available commercially for both oral and parenteral administration. It has rapid onset of action (1 to 4 days) because it does not require further activation by the kidneys. It increases the level of calcium (Ca^{++}) in the blood by increasing the absorption of calcium from intestine, by decreasing the excretion of calcium in urine and by enhancing the release of calcium into blood from bone. Calcitriol acts in concert with parathyroid hormone (PTH) in all three of these actions.

Calcitriol is metabolised to calcitroic acid through the action of 24-hydroxylase enzyme. Calcitroic acid is excreted in the urine. Calcitriol is prescribed for the treatment of hypocalcaemia, hypoparathyroidism, rickets, osteomalacia and renal osteodystrophy. It is also used in treatment of osteoporosis and prevention of corticosteroid-induced osteoporosis. The main adverse drug reaction associated with calcitriol therapy is hypercalcaemia. Calcitriol is more potent than vitamin D_3 (therefore, the dose is several hundred fold smaller than the vitamin D_3) and also clinical signs of toxicity resolve more readily. The drug is expensive and has limited use in domestic animals.

Dose

Dogs & cats : 2.5 – 3 ng (nanogram)/kg, PO, once daily.

Calcifediol

Calcifediol (Calcidiol, 25-hydroxycholecalciferol, 25-OH D3, or 25-hydroxyvitamin D) is the metabolic product of vitamin D_3. It is available commercially for oral use. After absorption, calcifediol is converted in the kidneys into calcitriol (1,25-$(OH)_2D_3$), the active form of vitamin D. It is thought to be important in reabsorbing calcium in renal tubules and regulating calcium flux in muscles. Therapeutic doses in veterinary species have not been established.

II. Synthetic Analogues of Vitamin D and Metabolites

Alfacalcidol

Alfacalcidol (1α-Hydroxycholecalciferol, 1-OH D_3) is a synthetic derivative of vitamin D_3 that is hydroxylated in the 1α-position. It is a pro-drug that is rapidly hydroxylated in the liver to 1,25-$(OH)_2$ D_3 or calcitriol. It has lesser effect on the calcium metabolism than calcitriol, while having more potent effects on the parathyroid hormone level and immune system, including regulatory T cells. Because alfacalcidol does not require renal hydroxylation at position 1 that is the limiting step in generation of the active form of endogenous vitamin D_3, it has been used to treat renal osteodystrophy or renal rickets (in renal rickets, conversion of 25-OH D_3 into 1,25$(OH)_2$ D_3 does not occur due to chronic renal disease). It is orally active and clinically equally effective on long-term basis to calcitriol.

In veterinary medicine, alfacalcidol is used clinically for the prevention of hypocalcaemia. It has also been used as poultry feed additive. When used as poultry feed additive, it prevents tibial dyschondroplasia and increases phytate bioavailability. Alfacalcidol is considered to be a more useful form of vitamin D supplementation, mostly due to much longer half-life and lower kidney load. It should not be used concurrently with other vitamin preparations. Hypercalcaemia should be watched for and therapy interrupted for few days when it develops.

Dose

For hypocalcaemia

Cattle : 0.35 mg (total), SC, 1 to 2 days before calving. It may be repeated only after 3 to 4 days, if calving has not occurred.

Dihydrotachysterol

Dihydrotachysterol (DHT) is an analogue of vitamin D_2 that is obtained synthetically by reduction of vitamin D_2 (therefore, it is also called DHT_2). Like vitamin D_2, it is activated in the liver but, unlike vitamins D_2 and D_3, does not require renal hydroxylation. Dihydrotachysterol is about 1/450 as active as vitamin D_2 in the antirachitic assay but it has a rapid onset of action (2 hours), a shorter half-life, and a greater effect on mobilising bone calcium than does vitamin D. Further, it does not require parathormone dependent activation in the kidney. Due to these reasons, DHT

is considered very useful in hypothyroidism and renal bone disease. Dihydrotachysterol is more expensive than vitamin D_2, but calcium levels are easier to control because it acts quickly and its side effects (e.g. hypercalcaemia) resolve more rapidly due to shorter action.

Dose

Dogs & cats : 0.03 – 0.06 mg/kg/day, PO. Dose may be decreased by 0.01 mg (total) every other day to 0.01 mg/kg/day.

Paricalcitol

Paricalcitol (19-Nor-1,25-$(OH)_2$-vitamin D_2, 19-nor-1,25-dihydroxyvitamin D_2) is a synthetic analogue of 1,25-dihydroxyergocalciferol, the active form of vitamin D_2. It is primarily used for the prevention and treatment of secondary hyperparathyroidism associated with chronic renal failure. Its exact mechanism of action is not clear, but it is suggested that paricalcitol, like 1,25-dihydroxyergocalciferol, acts as an agonist for the vitamin D receptor and lowers the blood parathormone level. Potential adverse events of paricalcitol are, in general, similar to those observed with excessive vitamin D intake.

Calcipotriol

Calcipotriol (Calcipotriene) is a synthetic derivative of calcitriol. It has been approved for the treatment of psoriasis, a chronic autoimmune disease that appears on the skin. The precise mechanism of calcipotriol in remitting psoriasis is not well-understood. Calcipotriol is commercially available as a cream, ointment or solution for dermal application.

Tacalcitol

Tacalcitol is a synthetic cholecalciferol (vitamin D_3) analogue. Tacalcitol is used topically and exerts its biological activity by preventing the proliferation of keratinocytes. It is primarily used to treat psoriasis. It has been used for vitiligo and some other dermal conditions.

III. Miscellaneous Agents

Several other preparations of vitamin D are available commercially. These include cod liver oil that contains not less than 85 I.U. antirachitic activity and halibut liver oil that contains about 3000 I.U. of antirachitic activity (see for detail vitamin A). Many vitamin and vitamin-mineral mixtures contain vitamin D.

VITAMIN E / TOCOPHEROLS

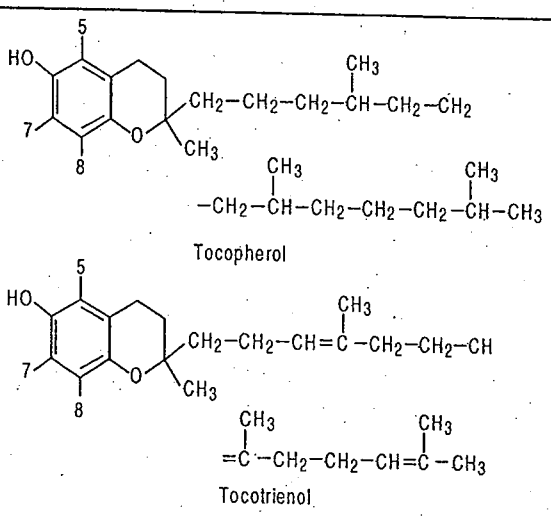
Vitamin E is an important group of fat-soluble vitamins. Originally, the term vitamin E was applied to α-tocopherol, an alcohol isolated from wheat germ oil and described as a factor required for improving reproductive performance (Greek word '*tokos*' meaning child birth or reproduction). Now the term vitamin E is used collectively for α-tocopherol and other tocol or tocotrienol derivatives possessing biological activity of α-tocopherol. In nature, vitamin E is present in growing plants and in cereals mainly as a mixture of various tocopherols in varying proportions. Vitamin E is unique among the vitamins as it acts primarily at the chemical level as an antioxidant and does not serve as cofactor in cellular regulation. One international unit (I.U.) of vitamin E is equivalent to the activity of 1 mg of dl-α-tocopherol acetate.

Chemistry and Source

Vitamin E is contained mainly in various plant oils (e.g. wheat germ, cotton-seed, palm and rice) and whole grain cereals, where it constitutes the non-saponifiable fraction. It is also present in some animal tissues like fat, liver, heart and pancreases, but animal products are a poor source of vitamin E.

In nature, vitamin E occurs in two major chemical forms, tocopherol and tocotrienol collectively called tocopherols. Both types contain a chromanol ring, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain which allows for penetration into biological membranes. Both the tocopherols and tocotrienols occur in alpha, beta, gamma and delta forms, determined by the number and position of methyl groups on the chromanol ring (Figure 54.6). Eight different vitamin E forms known as alpha-, beta-, gamma- and delta- tocopherol and alpha-, beta-, gamma- and delta-tocotrienol have been identified in plants. Out of all these naturally occurring tocopherols, the alpha-tocopherol (5,7,8-trimethyl tocol) is considered to be the most abundant and most important tocopherol. The d-isomer of tocopherol is more potent than the l-isomer.

The tocopherols as a group are insoluble in water, but soluble in organic solvents including fixed oils. All tocopherols are rapidly oxidised and destroyed when exposed to air and light.



Type of tocopherol (Isomer)	Primary structure	Position of methyl group
1. Alpha-tocopherol	Tocopherol	5, 7, 8
2. Beta -tocopherol	Tocopherol	5, 8
3. Gamma-tocopherol	Tocopherol	7, 8
4. Delta-tocopherol	Tocopherol	8
5. Alpha-tocotrienol	Tocotrienol	5, 7, 8
6. Beta-tocotrienol	Tocotrienol	5, 8
7. Gamma-tocotrienol	Tocotrienol	7, 8
8. Delta-tocotrienol	Tocotrienol	8

Fig. 54.6. Chemical structures of naturally occuring tocopherols (Vitamin E).

Absorption and Fate

Vitamin E is absorbed from intestine by a mechanism probably similar to that for other fat soluble vitamins. It enters the blood

stream in chylomicrons via the lymph. It circulates in plasma in association with β-lipoprotein and is distributed to all tissues. Vitamin E enters cells by processes similar to those for low density lipoproteins (LDL) uptake. The LDL uptake membrane receptors through receptor-mediated endocytosis appear to be responsible for vitamin E uptake. Once in cells, vitamin E is incorporated into lipid membranes.

Vitamin E is stored in tissues, mainly in the liver and adipose tissue and excreted slowly in the bile and urine as metabolites. The urinary metabolites are glucuronide of tocopheronic acid and its lactone. Several other metabolites with quinone structure have been found in tissues.

Physiological Functions

Vitamin E is very reactive and has many biological functions. The predominant role of vitamin E (tocopherols) appears to be as an antioxidant. In acting as an antioxidant, vitamin E presumably prevents oxidation of essential cellular constituents or prevents formation of toxic oxidation products. Primarily, vitamin E prevents the antioxidation of highly unsaturated fatty acids when they are exposed to molecular oxygen, thereby prolonging their biological life. In the absence of vitamin E, the quantity of unsaturated fatty acids in cells becomes diminished causing abnormal structure and function of some cellular organelles as mitochondria, lysosomes and cell membranes. As unsaturated fatty acids constitute an important part of biological membranes, the antioxidant action of vitamin E has been equated with protection and stabilisation of cell membranes.

Vitamin E prevents formation of toxic oxidation products such as free radical and hyperperoxides from unsaturated fatty acids. The quinone moiety of vitamin E is capable of quenching free radicals such as free radicals of hydrogen ($H^{\bullet}$), superoxide radicals ($O_2^{\bullet}$), hydroxyl radicals ($OH^{\bullet}$), and other lipid-derived radical species ($LOO^{\bullet}$). In the course of its action as a free radical scavenger, vitamin E is sacrificed. The decrease in peroxide formation from unsaturated fatty acids also has a stabilising effect on cell membranes containing unsaturated fatty acids. Vitamin E and selenium enhance each other's action and together protect cells against harmful build-up of peroxides.

In addition to anti-oxidant actions, vitamin E is reported to potentiate the immune mechanism of body and increases resistance to bacterial and viral infections. Vitamin E is also useful in enhancing the absorption of vitamin A from the GI tract and elevating its concentration in liver and other tissues. This effect may be related mainly to the protection of vitamin A by antioxidant properties of vitamin E. Vitamin E also has a regulatory effect on enzymatic activities (e.g. protein kinase C) and an effect on gene expression. It plays a role in neurological functions and inhibition of platelet aggregation.

Disorders of Vitamin E

Deficiency of Vitamin E

Deficiency of vitamin E occurs most commonly in young rapidly growing ruminants (e.g. calves and lambs) receiving vitamin E deficient diets. Any stored feed that has significant quantities of unsaturated fats can be considered depleted of vitamin E and, therefore, potentially disease producing. Naturally occurring deficiencies are rare in adult animals.

Signs of vitamin deficiency in animals include structural and functional abnormalities of many organs and organ systems, mainly muscle weakness and degeneration. The principal vitamin E deficiency disease of cattle and sheep is muscular dystrophy or white muscle disease characterised by stiff, swollen and painful muscles. Both skeletal and cardiac muscles are susceptible causing a stiff gait, inability to stand (due to skeletal muscle weakness) and sudden death (due to cardiac failure). Pale white areas of degeneration can be seen is skeletal muscle following necropsy. In poultry, vitamin E deficiency causes decreased hatchability of eggs because of embryonic death. Young chicks develop an encephalomalacia and exudative diathesis. In carnivores and omnivores, the vitamin E deficiency is brought about by high levels of unsaturated fats (diets from fish by-products) which destroy the naturally occurring vitamin E. Primary lesions are yellow brown pigment deposition in fat and inflammatory changes in adipose tissues (yellow fat disease). In horses, equine degenerative myeloencephalopathy (EDM) or equine motor neurone disease (EMND) is believed to be associated with vitamin E deficiency. In lower animals, lack of vitamin E can cause degeneration of the germinal epithelium in testes and, therefore, male sterility (hence vitamin E is sometimes referred to as anti-sterility vitamin). Lack of vitamin E can also lead to resorption of foetus after conception in the female.

Nutritional requirement for vitamin E are not clearly defined because the availability of vitamin is conditioned by the composition of diet. In general, prevention of vitamin E deficiency requires a daily intake of approximately 150 mg of vitamin E for calves and 25 mg for lambs. Any diet that contains significant amount of fish oil or other unsaturated oils may require additional vitamin E.

Excess of Vitamin E

Hypervitaminosis E does not occur naturally in animals. Very high dose of vitamin E may produce abdominal cramps, diarrhoea and lethargy in human beings and animals.

PHARMACOLOGY OF VITAMIN E

Alpha Tocopherol

Alpha-Tocopherol (α-Tocopherol, 5,7,8-trimethyl tocol; α-T) is a methylated tocol that exhibits the most vitamin E activity of tocopherols. It is a light yellow, viscous, odourless and oil liquid that deteriorates on exposure to light. It is obtained from wheat germ oil or prepared synthetically. The main naturally occurring substance is d-alpha-tocopherol and principal compounds used commercially are dl-alpha-tocopherol, d-alpha-tocopherol acetate (d-alpha tocopheryl acetate) and d-alpha-tocopherol succinate (d-alpha tocopheryl acid succinate). Tocopheryl nicotinate and tocopheryl linolate esters are also used in cosmetics and some pharmaceuticals. The esters are preferred for therapeutic use because of their greater stability. The dl-alpha-tocopherol is available for oral and parenteral administration.

Vitamin E is mainly recommended for the prevention and treatment of various vitamin E deficiency related conditions. Vitamin E is also widely used in supplements and diets for its antioxidant properties. It is often combined with selenium for both prophylactic and therapeutic uses. Vitamin E is also available with vitamins A and D and in multi-nutrient supplements. Vitamin E displays no significant adverse effects even at very large doses. Abdominal cramps, loose motions and lethargy in human beings have been described as side effects of extremely large doses of α-tocopherols.

Dose

Vitamin E

Dogs	:	100 – 500 I.U. (total), PO, once daily.
	:	70 I.U. (total), IM, intermittently.
Cats	:	10 – 20 I.U./kg, PO, 2 times daily.

Tocopherol acetate

Dogs	:	100 – 500 mg/day, PO.
Cats	:	100 mg/day, PO.
Calves	:	300 – 2000 mg/day, PO.
Lambs	:	100 – 500 mg/day, PO.

Note: 1 mg of dl-alphatocopherol acetate contains 1 I.U. of vitamin E, 1 mg of d-alpha tocopherol acetate contains 1.36 I.U. of vitamin E, 1 mg of d-alpha tocopherol succinate contains 1.21 I.U. of vitamin E, and 1 mg of d-alpha tocopherol contains 1.49 I.U. of vitamin E.

Tocofersolan

Tocofersolan (Tocophersolan) is a synthetic polyethylene glycol derivative of α-tocopherol. Unlike the natural forms of vitamin E, tocofersolan is water soluble and hence is used as a vitamin E supplement or to treat vitamin E deficiency in individuals who cannot absorb fats due to digestive malabsorption or disease. Tocofersolan is also used in cosmetics and pharmaceuticals as an antioxidant.

VITAMIN K

Vitamin K (K for koagulation/coagulation) is a group of naphthoquine compounds essentially required for the biosynthesis of several blood clotting factors. Vitamin K occurs in nature in two forms – vitamin K_1 and vitamin K_2. A primary deficiency of vitamin K in domestic animals does not occur under normal conditions due to the availability of vitamin from natural sources.

Chemistry and Source

Vitamin K are 2-methyl-1,4-naphthoquinone derivatives. All members of the vitamin K group of vitamins share the methylated naphthoquinone ring structure, and vary in the aliphatic side chain attached at the 3-position. The side chain in vitamin K_1 is phytyl and in vitamin K_2 is prenyl.

Vitamin K_1/Phytomenadione: Vitamin K_1 (Phytomenadione, phytonadione, phylloquinone or phyllomenaquinone) is 2-methyl-3-phytyl-1,4-naphthoquione. It invariably contains in its side chain four isoprenoid residues, one of which is unsaturated. Vitamin K_1 is present in plants and mainly concentrated in the chloroplasts of plant leaves. Green leafy vegetables, lucerne, horse chestnut leaves etc. are good source of vitamin K_1.

Vitamin K_2/Menaquinones: Vitamin K_2 is not a single compound but is a series of substances (menaquinones) with varying lengths of side chains. In vitamin K_2, the phytyl side chain of vitamin K_1 is replaced by a side chain containing 2 to 13 prenyl units (Figure 54.7). The number of prenyl unit varies lipophilicity for the different function of intestinal absorption, transport, tissue distribution and bio-availability. Large amounts of vitamin K_2 is synthesised by intestinal and ruminal bacteria, including the conversion of K_1 to K_2 by bacteria in the small intestines. Vitamin K_2 is also derived from rotted fish.

Meat and animal products contain little vitamin K.

Absorption and Fate

Vitamins K_1 and K_2 are absorbed from intestine via lymph and require fat and bile salts for absorption. Vitamin K_1 is absorbed by an energy dependent active transport mechanism in the jejunum, whereas vitamin K_2 is absorbed by simple diffusion in the distal portion of small intestine and in colon. Dietary vitamin K is transported to liver by chylomicrons and intestinal very-low-density lipoprotein (VLDL) particles and from liver by VLDL and LDL. Vitamin K is only temporarily stored in liver; very little vitamin K accumulates in other tissues. In contrast to the slower turnover of other fat-soluble vitamins (week to months), the total pool of vitamin K in the body is replaced rapidly (hours to days).

Vitamin K is metabolised rapidly to the more polar metabolites, which are excreted in bile and urine. Metabolism mainly occurs in liver by side chain cleavage and glucuronide conjugation.

Physiological Functions

Vitamin K is essentially required for the hepatic biosynthesis of clotting factors II (prothrombin), VII, IX and X, all of which are important in blood coagulation. Vitamin K primarily functions as a cofactor for a microsomal enzyme system that activates the precursors of these clotting factors. The mechanism of action of vitamin K and role of these factors are discussed in Chapter 35.

Disorders of Vitamin K

Deficiency of Vitamin K

A primary deficiency of vitamin K under natural conditions occurs rarely in domestic animals because of high content of vitamin K in most plants and substantial synthesis of vitamin K substances by microbes in the GI tract. However, vitamin K deficiency may occur in animals when impairment of the flow of bile reduces digestion and absorption of the vitamin. Naturally occurring vitamin K deficiency may occur in poultry under intensive management, where they do not get green plants. Vitamin K deficiency in animals may also occur with long-term antimicrobial therapy (that alters intestinal flora), liver diseases, malabsorption, and exposure to mouldy sweet clover or oral anticoagulant rodenticides (e.g. warfarin). High intake of vitamin E may act as vitamin K antagonist.

The most important clinical manifestations of vitamin K deficiency is bleeding tendency due to lowering of the levels of prothrombin and other clotting factors in blood. Epistaxis, haematuria, ecchymoses, gastrointestinal bleeding and post-operative haemorrhage are common.

The dietary requirement of vitamin K for many animals is difficult to establish because it depends on several factors including synthesis of vitamin by intestinal bacteria, exposure to green plants and extent to which some animal species practice coprophagy (some species take vitamin K through faeces). In general, birds tend to have relative high requirements for vitamin K, while ruminants do not need an external source. It has been suggested that small animals should obtain approximately 0.5-1 mg as phylloquinone per kg diet.

Excess of Vitamin K/Hypervitaminosis K

Vitamin K_1 and K_2 are virtually non-toxic to animals, even when given in large amounts. The synthetic forms of vitamin K (e.g. vitamin K_3), however, have shown toxicity.

PHARMACOLOGY OF VITAMIN K

Vitamin K is used therapeutically in the prophylaxis and treatment of bleeding disorders due to deficiency of vitamin K-dependent blood clotting factors. In veterinary medicine, vitamin K is predominantly used as haemostatic agent in the oral anticoagulant rodenticides and sweet clover poisoning.

Classification

Commercially, vitamin K is available as phytomenadione (vitamin K_1), the natural plant form and menadione (vitamin K_3) and its derivatives, the synthetic forms. The menaquinones (vitamin K_2) have limited use in therapeutics.

I. Natural vitamin K compounds
   e.g. phytomenadione and menatetrenone.
II. Synthetic vitamin K compounds
   1. Menadione
   2. Derivatives of menadione
      e.g. acetomenadione, menadione sodium bisulphite and menadione sodium diphosphate

I. Natural Vitamin K Compounds

Phytomenadione/Vitamin K_1

Phytomenadione (Phytonadione, phylloquinone) or vitamin K_1 is a lipid soluble naturally occurring vitamin K. It is the most commonly used vitamin K preparation in both human and veterinary medicines. Phytomenadione is available in several formulations for oral and parenteral administration. Following IM injection, phytomenadione is readily absorbed.

In animals, phytomenadione is used mainly for the treatment and/or prevention of bleeding due to overdosage of oral anticoagulant drugs (e.g. warfarin and coumarin). In oral anticoagulants poisoning, phytomenadione is considered as the preparation of choice because it acts more rapidly than the synthetic vitamin K preparations. Phytomenadione is usually administered for 7 days in case of warfarin poisoning, but treatment may need to be continued for several weeks (4 to 8 weeks) in case of long acting oral anticoagulant rodenticides (e.g. brodifacoum, bromadiolone and difenacoum) poisoning. Dose of vitamin K_1 depends on the severity of hypoprothrombinaemia. Prothrombin time is usually monitored for 2 days after phytomenadione is discontinued to detect residual rodenticide toxicity.

In animals, phytomenadione is administered mainly by oral, IM or SC route. For SC injection in large animals, each dose should be divided and administered in multiple sites. In severe cases, the drug may be given initially by slow IV injection. However, severe reactions resembling anaphylaxis may occur following its IV administration.

Dose

For anticoagulant toxicity

Dogs & cats	:	2.5 – 5 mg/kg/day, IM or SC (several sites) in divided doses for 1 to 3 days followed by oral administration. For severe cases, initial treatment may be given by slow IV injection.
	:	0.25 – 2.5 mg/kg/day, PO, in 2 to 3 divided doses.
Horses, cattle, sheep, goats, and pigs	:	0.5 – 1 mg/kg/day, IM or SC (several sites) in divided doses.

Menatetrenone

Menatetrenone (Menaquinone-4) is one the several forms of menaquinone, usually referred to as vitamin K_2. Menatetrenone has been reported to be the most potent form of vitamin K_2. Similar to phytomenadione, it helps in synthesis of various coagulation proteins. It has also shown efficacy in preventing bone resorption by inducing apoptosis of osteoclasts and by inhibiting formation of osteoclasts. Accordingly, it is used as a haemostatic agent and as adjunctive therapy for the pain of osteoporosis.

II. Synthetic Vitamin K Compounds

1. Menadione

Menadione (Menadiol, menaphthone) or vitamin K_3 is a synthetic form of vitamin K, which does not have aliphatic side chain. It is an analogue of 1,4-naphthoquinone with a methyl group in the 2-position. The menadione is lipid soluble and is practically insoluble in water. It is about 3 times more active than natural phytomenadione, but has slower onset of action. Menadione is also called a vitamin precursor or a pro-vitamin, as it can be converted in the liver to active menaquinones (Vitamin K_2) by chemical alkylation *in vivo*.

Unlike vitamin K_1 and K_2, menadione and its derivatives are absorbed from the GI tract even in the absence of bile. Absorption occurs slowly by diffusion in the distal portion of small intestine and in the colon. Following IM injection, synthetic vitamin K preparations are absorbed more readily than the oral route. Menadione is metabolised rapidly (30-40 minutes, after IV administration) mainly to diol (hydroquinone) form and excreted as glucuronide and sulphate conjugates.

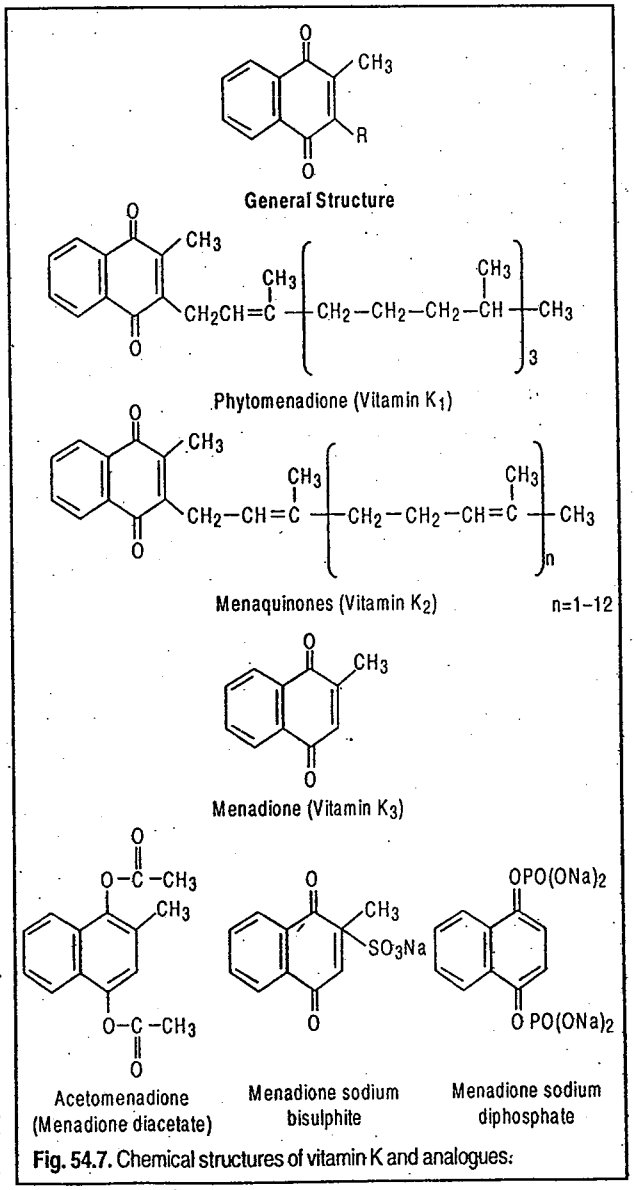


Fig. 54.7. Chemical structures of vitamin K and analogues.

Menadione is less used in veterinary medicine because its action is slow (mainly due to slow absorption) to be used effectively in acute conditions (e.g. oral anticoagulants poisoning). However, it can be used for chronic therapy once the acute crisis has been resolved. Menadione is irritating to the skin and all mucous membranes with its alcoholic solution becoming almost vesicant. In human beings, menadione and its derivatives have been implicated in producing haemolytic anaemia, hyperbilirubinaemia and kernicterus in the newborn, especially in premature infants. It is not used directly as a nutritional supplement due to high toxicity and low stability.

2. Derivatives of Menadione

Several synthetic derivatives of menadione (vitamin K_3) are available commercially. These include the lipid soluble derivative acetomenadione (acetomenaphthone; menadione diacetate; vitamin K_4) and the water-soluble derivatives menadione sodium bisulphite and menadione sodium diphosphate (menadione tetrasodium diphosphate). Menadione sodium bisulphite (MSB) has limited stability to light, heat, humidity, ambient pH values above neutrality and in the presence of some salts. Menadione dimethyl pyrimidinol bisulfite (MPB) and menadione nicotinamide bisulfite (MNB) are other synthetic forms which have higher stability but low solubility in water than MSB.

The derivatives of menadione are converted in the body to the active menadione. The water-soluble salts do not require bile for their intestinal absorption. Acetomenadione is used only by oral administration, while water-soluble menadione sodium diphosphate is used by both oral and parenteral routes. Like parent compound (menadione), lipid and water-soluble derivatives of menadione are not much useful in acute oral anticoagulant poisoning.

Dose

Acetomenadione

Dogs : 2 – 10 mg (total), PO, daily.

Horses & cattle: 100 – 400 mg (total), PO, daily.

Menadione sodium bisulphite

Poultry : 1 – 2 g/tonne of feed.

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CHAPTER 55

WATER SOLUBLE VITAMINS

Vitamin B complex
- Thiamine/Vitamin B_1
  - Properties
  - Chemistry
  - Absorption and fate
  - Mechanism of action and physiological functions
  - Disorders of thiamine
    - Deficiency of thiamine
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    - Classification
    - Natural vitamin B_1 compounds
    - Derivatives of thiamine
- Riboflavin/Vitamin B_2
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Vitamin C/Ascorbic acid
- Chemistry and source
- Absorption and fate
- Physiological functions
- Disorders of vitamin C
  - Deficiency of vitamin C
  - Excess of vitamin C
- Pharmacology of ascorbic acid

Water-soluble vitamins are a functionally diverse group of water-soluble micronutrients required by all animals. In most cases water-soluble vitamins are ingested as a natural dietary constituent and in some cases they may be synthesised by gastrointestinal microorganisms. Unlike fat-soluble vitamins, most water-soluble vitamins serve as precursors for enzyme cofactors, which help enzymes to act as catalysts in various metabolic reactions. In general, water-soluble vitamins are of little therapeutic importance in veterinary medicine because pure deficiencies of individual vitamins occur rarely in animals.

Most water-soluble vitamin therapies can be considered as prophylactic or supportive in nature.

VITAMIN B COMPLEX

The vitamin B complex comprises a large number of biological compounds which differ extensively in chemical structure and biological action. Traditionally, vitamin B complex comprises of 12 members namely thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, cyanocobalamin, choline, inositol, carnitine and p-aminobenzoic acid. However, in recent times only first eight of the above mentioned water soluble vitamins are included in the vitamin B complex. These agents are grouped in a single class because they originally were isolated from the same source, notably liver and yeast, and thought to be a single vitamin, referred to as vitamin B. The B complex vitamins are widely distributed in both plants and animal feed.

THIAMINE / VITAMIN B_1

Thiamine (Thiamin, aneurin) or Vitamin B_1 was the first member of the vitamin B complex to be identified, isolated and purified. Thiamine is water-soluble and heat labile. It occurs naturally in plants, meat, fish, eggs, etc. Yeast and wheat germ are the most concentrated natural sources. Thiamine is formed also in rumen by ruminal microorganisms hence ruminants do not require an additional dietary source. Its phosphate derivatives are involved in many cellular processes.

Properties

Thiamine is soluble in water, methanol and glycerol and practically insoluble in acetone, ether, chloroform and benzene. It is stable at acidic pH, but is unstable in alkaline solutions. Thiamine is unstable to heat, but stable during frozen storage.

Chemistry

Thiamine is a colourless compound with a chemical formula $C_{12}H_{17}N_4OS$. Thiamine contains a pyrimidine and a thiazole nucleus linked by a methylene bridge (Figure 55.1). There are five known natural thiamine phosphate derivatives: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), also commonly called thiamine pyrophosphate (TPP), thiamine triphosphate (ThTP, TPPP), and the recently discovered adenosine thiamine triphosphate (AThTP) and adenosine thiamine diphosphate (AThDP). Out of these thiamine phosphate derivatives, only two forms viz. thiamine pyrophosphate and thiamine triphosphate function as co-factors and have known physiological roles. The thiamine triphosphate predominates in neural tissues and brain.

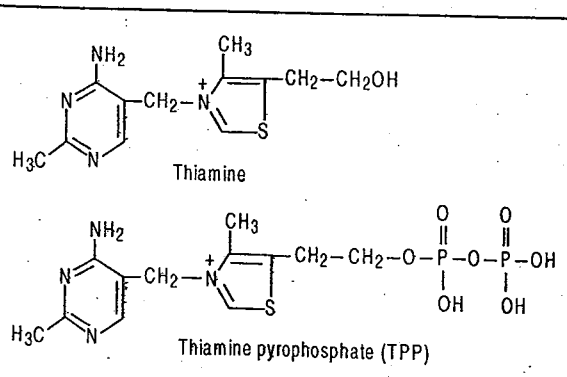


Fig. 55.1. Chemical structures of thiamine and its coenzyme thiamine pyrophosphate.

Absorption and Fate

Dietary thiamine is readily absorbed from the GI tract mainly by Na^+-dependent active transport mechanism. Absorption by passive diffusion may also occur, particularly at high thiamine concentration. After absorption, majority of thiamine in serum is bound to proteins, mainly albumin and transported to various organs. Thiamine is converted to its coenzyme forms in the liver. Like other water-soluble vitamins, thiamine is stored only to little extent in liver, kidneys, brain and some other tissues. Metabolism of thiamine occurs mainly by degradation of thiamine nucleus to pyrimidine. Excretion of thiamine depends on its body requirements and concentrations in the diet. At low concentration little or no thiamine is excreted, but when intake exceeds the minimal requirement, excess is excreted in urine as intact thiamine or as pyrimidine metabolite.

Mechanism of action and physiological functions

Thiamine is primarily the transport form of the vitamin, while the active forms are phosphorylated thiamine derivatives. The thiamine in cells occurs either in the pyrophosphate (diphosphate) or the triphosphate form. Thiamine pyrophosphate functions in carbohydrate metabolism as a coenzyme in the decarboxylation of α-keto acids, designated as transketolase reaction. In the tricarboxylic acid (TCA) cycle, thiamine pyrophosphate helps decarboxylation of pyruvic acid to acetyl CoA and α-ketoglutarate to succinyl CoA. In addition to above reactions, thiamine pyrophosphate facilitates transformation of 'ketols' (ketose phosphates) in the pentose phosphate pathways. In this pathway, NADP is reduced to NADPH, an essential reducing agent for synthetic reaction. In thiamine deficiency, metabolism of carbohydrates gets impaired because of defective TCA cycle regulation. In addition, disturbances in the pentose phosphate related carbohydrates pathways lead to decreased production of NADPH, which may affect synthetic processes such as fatty acid biosynthesis.

Thiamine triphosphate is proposed to be involved in sodium gating processes in neural tissues and brain. This aspect of thiamine metabolism may be related to the psychosis and impairment of neuromuscular control which are observed in humans with thiamine deficiency.

Disorders of Thiamine

Deficiency of thiamine

Thiamine deficiency may occur in animals as a result of inadequate dietary intake or destruction of the vitamin by either excessive cooking/heating during processing of diets for carnivores or presence of thiamine-destroying agents in feed or fodder. Thiamine deficiency of dietary origin (called primary thiamine deficiency) is not a common problem under natural conditions because most plants, especially seeds, yeast and milk contain adequate amounts of thiamine. The principal cause of thiamine deficiency in animals is the presence of enzyme thiaminase in diet, which destroys thiamine (called secondary thiamine deficiency). Thiaminase is abundant in the flesh of some fish, particularly spoiled fish, and plants like bracken fern (*Pteridium aquilinum*) and horsetail fern (*Equisetum arvense*). In ruminant species, particularly in growing sheep, thiamine

deficiency may occur following increased thiaminase activity in the rumen. Changes in diet, recent anthelmintic treatment, or other factors may disturb the rumen microflora with proliferation of thiaminase producing bacteria. High levels of tannins also reduce the bioavailability of thiamine in foodstuffs.

Deficiency of thiamine results mainly in loss of energy, a build up of pyruvic and lactic acids in tissues and decreased absorption of glucose. Tissues dependent on glucose or lactate-pyruvate for energy such as brain and heart are particularly compromised in thiamine deficiency. The main symptoms of thiamine deficiency are muscular incoordination, especially of hind legs, paralysis with head turned over back (opisthotonus), convulsions, diarrhoea and vomiting (in carnivores). In both herbivores and simple stomach animals, predominant characteristics of thiamine deficiency are polioencephalomalacia. In humans, thiamine deficiency referred to as 'beriberi' is characterised by polyneuritis, cardiac vascular symptoms and gastrointestinal disorders.

The requirement for thiamine is related to metabolic rate and is greatest when carbohydrates are the source of energy. As a general requirement, animals should receive from 4 to 10 mg of thiamine per kg dry food. The daily requirement of thiamine for monogastric animals is about 30-60 µg/kg body weight. The addition of yeast, cereals, grains, meat meal to the ration usually provides adequate thiamine.

Excess of thiamine

No known disorder from excess intake of thiamine has been reported in animals.

PHARMACOLOGY OF THIAMINE

Thiamine deficiency can be treated by supplementing thiamine in the diet. In acute deficiency, thiamine may be administered either by oral or parenteral routes.

Classification

I. Natural vitamin B_1 compounds
   e.g. thiamine.

II. Derivatives of vitamin B_1
   e.g. benfotiamine, sulbutiamine, allithiamine, prosultiamine and fursultiamine.

I. Natural Vitamin B_1 Compounds

Thiamine

Thiamine or vitamin B_1 is commercially available as thiamine hydrochloride and its main source is synthetic. Thiamine hydrochloride is a white crystalline powder that is very soluble in water and glycerine, but not in alcohol, ether or other organic solvents. Thiamine is practically devoid of pharmacological actions when given in usual therapeutic doses. Its mechanism of action and pharmacological effects in body are essentially similar to those observed with natural thiamine.

In veterinary medicine, thiamine HCl is used mainly for the treatment of bracken fern poisoning in horses and pigs; cattle and sheep respond less to thiamine. Successful treatment of secondary thiamine deficiency depends on the severity of condition and time of institution of vitamin therapy. Parenteral administration of thiamine shortly after the onset of neurological signs gives better results. Removal of the antivitamin source (e.g. thiaminase containing diet) is essential for successful therapy. Prophylactic parenteral administration of thiamine may be of value in herd outbreaks of polioencephalomalacia. Amprolium (a structural analogue of thiamine) induced thiamine deficiency in small animals can be treated by adding the vitamin to the diet.

Thiamine is virtually free of adverse effects. Isolated diurnal case of toxic reactions to the long-term parenteral administration of thiamine producing hypersensitivity has been reported in human medicine. Rare reports of anaphylaxis caused by high-dose thiamine injections into the vein or muscle have been documented.

Dose

For treatment of thiamine deficiency

Dogs & cats	:	100 – 250 mg (total), SC, 2 times daily until regression of symptoms.
Cats	:	10 mg/kg, PO, once daily for 3 weeks.
	:	50 mg (total), IM, 1 to 2 times daily.
Cattle & sheep	:	5 – 10 mg/kg, IM or slow IV. Dose may be repeated every 3 to 4 hours, if required. The initial dose is usually given IV followed by IM injections for 2 to 4 days.
Horses & pigs	:	0.25 – 1.25 mg/kg, IM or slow IV, twice daily for up to 7 days.

II. Derivatives of Thiamine

Thiamine derivatives with improved pharmacokinetics have been developed and are considered more effective in alleviating the symptoms of thiamine deficiency and other thiamine-related conditions. These compounds include benfotiamine, sulbutiamine, allithiamine, prosultiamine and fursultiamine.

Benfotiamine

Benfotiamine (S-benzoylthiamine O-monophoshate) is a synthetic S-acyl derivative of thiamine (vitamin B_1). After absorption, benfotiamine can be dephosphorylated by cells to the lipid-soluble S-benzoylthiamine. It is believed that treatment with benfotiamine leads to increased intracellular thiamine diphosphate levels, which perform the pharmacological functions of the drug. Benfotiamine is considered effective for the treatment of diabetic retinopathy, neuropathy and nephropathy.

Sulbutiamine

Sulbutiamine is a synthetic derivative of thiamine (vitamin B_1). It contains a dimer of two modified thiamine molecules, which makes it lipid soluble. Due to the high lipid solubility it easily crosses blood brain barrier and reaches the brain in high concentration. In the brain it is metabolised to thiamine and thiamine phosphate esters. Sulbutiamine is used primarily in human medicine for the treatment of asthenia, which is manifested by lack of muscle strength, malaise, dizziness or fatigue. Sulbutiamine has few reported adverse effects at therapeutic dosages, but may cause mild skin allergy and agitation.

Allithiamine

Allithiamine (Thiamine allyl disulfide) is a lipid-soluble form of vitamin B_1. It has better oral bioavailability and distribution than the water-soluble form of vitamin B_1. It is used for the treatment of various conditions related to thiamine deficiency.

RIBOFLAVIN / VITAMIN B_2

Riboflavin (Riboflavine, lactoflavin) or Vitamin B_2 is a water soluble B vitamin initially identified and isolated from milk and

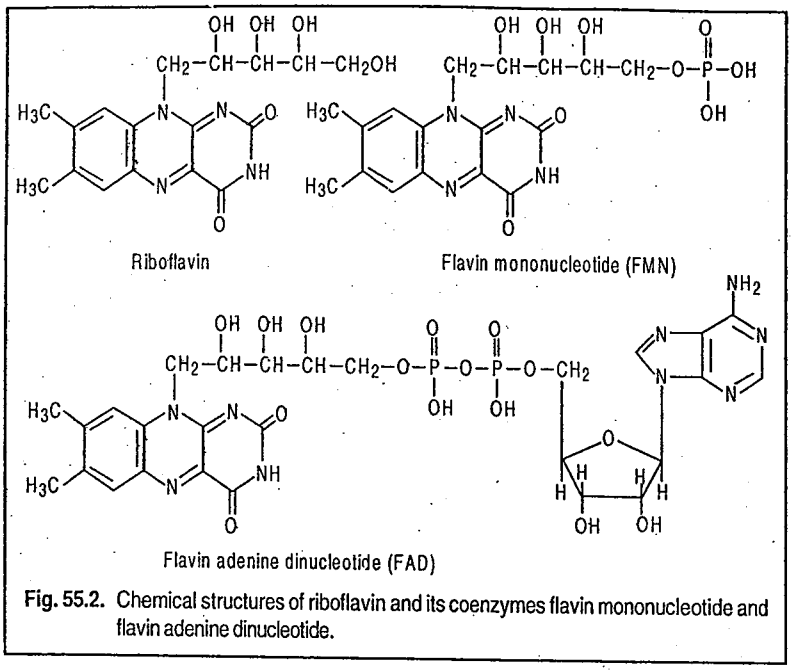


Fig. 55.2. Chemical structures of riboflavin and its coenzymes flavin mononucleotide and flavin adenine dinucleotide.

some other sources. Riboflavin is a part of the structure of isoalloxazine nucleotides, which are coenzymes for the flavodehydrogenases. Riboflavin is required for a wide variety of cellular processes including metabolism of fats, ketone bodies, carbohydrates and proteins.

Chemistry and source

Riboflavin is a ribose containing structure (therefore called riboflavin). It combines in tissues with phosphoric acid to form two coenzymes, flavin mononucleotide (riboflavin phosphate, riboflavin mononucleotide) and flavin adenine dinucleotide (riboflavin dinucleotide). Flavin mononucleotide (FMN) consists of one molecule of riboflavin and one of phosphoric acid, while flavin adenine dinucleotide (FAD) contains one molecule of riboflavin, one of adenine and two of phosphoric acid (Figure 55.2).

Riboflavin is yellow or yellow-orange in colour. It is synthesised by all plants and many microorganisms, but not by higher animals. Riboflavin is present in milk and milk products, meat, eggs, fish roes and to a lesser degree in fruits and vegetables. Yeast extract is considered to be exceptionally rich in vitamin B_2, and liver and kidney are also rich sources.

Absorption and Fate

Riboflavin is rapidly absorbed from the upper gastrointestinal tract by a specific transport mechanism and is distributed to all tissues in association with albumin. In the intestine and in other tissues, riboflavin is converted to FMN by enzyme flavokinase. Like thiamine, little storage of riboflavin occurs in the body organs like liver, kidneys and heart, though small quantities exist in practically all organs and tissues. Excretion of riboflavin depends on its concentration in diet and body requirements i.e. small amount is excreted if ingestion is about the minimum daily requirements, but as intake of vitamin is increased a large portion is excreted unchanged. Urine is the major route of excretion for riboflavin, although some FAD is also excreted in bile. In most animals, the half-life of riboflavin is several weeks to several months as it is covalently bound to certain enzymes such as succinic dehydrogenase.

Physiological functions

The FMN and FAD, the physiological active forms of riboflavin, function as prosthetic groups of oxidation-reduction enzymes known as flavoenzymes or flavoproteins. These enzymes function in the oxidative degradation of pyruvate, fatty acids and amino acids and also in the process of electron transport. The FMN is mainly associated with various flavoenzymes including cytochrome reductase and L-amino acid oxidase, while FAD is coenzyme to D-amino acid oxidase, xanthine oxidase and aldehyde oxidase. FAD is also required to the production of pyridoxic acid from pyridoxal (vitamin B_6) and to convert retinal (vitamin A) to retinoic acid. Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) is also FAD dependent. Riboflavin participates in cytochrome P-450 metabolism (dehydrogenation, hydroxylation, oxidative decarboxylation, reduction, etc.).

Disorders of Riboflavin

Deficiency of riboflavin (Ariboflavinosis)

Although riboflavin is essential for cellular oxidative processes in all animals, the occurrence of riboflavin deficiency under natural conditions is rare in farm animals because it is readily available from green plants and also synthesised by gut microflora. Deficiency of riboflavin is sometimes seen in simple stomach animals and pre-ruminating ruminants. The deficiency of riboflavin is called primary when diet is deficient in vitamin B_2 and secondary when diet is sufficient in vitamin but its concentration in body is low either due to defective absorption in intestine, improper usage in body or enhanced elimination from body. Deficiency of riboflavin is usually associated with increased lipid-peroxidation and altered metabolisms of other B vitamins (e.g. folic acid, pyridoxine and nicotinic acid) and vitamin K.

Riboflavin deficiency signs include lesions of oral cavity and lips, stomatitis, glossitis and seborrhoeic dermatitis. In severe form of riboflavin deficiency, the filiform papillae of tongue are lost and the tongue changes colour from its usual pink to magenta. Other signs those may be observed in some species include slow growth, alopecia, abnormal haematopoiesis with anaemia, decreased fertility and vascularisation of eye with pain, irritation and ocular discharge. Curled toe paralysis of young chicks, reduced egg yield in adults and reduced hatchability in breeding hens have been observed in riboflavin deficiency in poultry.

Turnover of riboflavin is usually dependent on the turnover of the proteins to which it is associated. In general, most small animals require 2-6 mg riboflavin per kg diet. Daily requirements for pigs are 60-80 µg/kg body wt; 2-3 g/tonne of feed provides adequate vitamin supplement.

Excess of riboflavin

In animals, there is no evidence for riboflavin toxicity produced by excessive intake in diets.

PHARMACOLOGY OF RIBOFLAVIN

Riboflavin/Vitamin B_2

Riboflavin is an orange-yellow crystalline powder, which has slightly bitter taste and a slight odour. Although riboflavin can be extracted from yeast, but commercial vitamin is mostly obtained from synthetic source. Oral or parental administration of riboflavin does not produce any overt pharmacological effect.

The only established therapeutic use of riboflavin in veterinary and human medicines is to treat or prevent disease caused by its deficiency. In human medicine, riboflavin supplements have been used as part of the phototherapy treatment of neonatal jaundice. High dose riboflavin either alone or along with beta-blockers is considered useful in the prevention of migraine. In general, the deficiency of riboflavin occurs mostly in association with deficiency of a number of other vitamins as well as nutritive factors. Accordingly, riboflavin is often used clinically as a part of multivitamin therapy.

Dose

Dogs : 10 – 20 mg/day, PO.

Cats : 5 – 10 mg/day, PO.

NICOTINIC ACID / VITAMIN B_3

Nicotinic acid (Niacin) or Vitamin B_3 is a water-soluble B vitamin, which is widely distributed in nature. Nicotinic acid or Niacin is referred to as vitamin B_3 because it was the third of the B vitamins to be discovered. Deficiency of nicotinic acid leads to the disease pellagra in man and black tongue in dogs hence nicotinic acid is also called anti-pellagra or anti-black tongue factor.

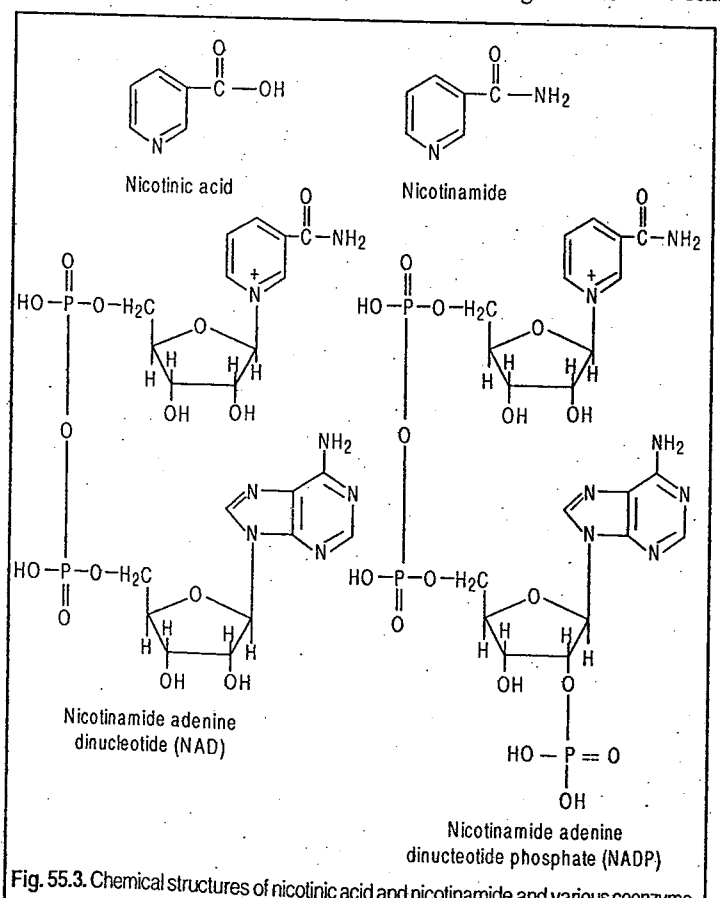


Fig. 55.3. Chemical structures of nicotinic acid and nicotinamide and various coenzyme forms of nicotinic acid.

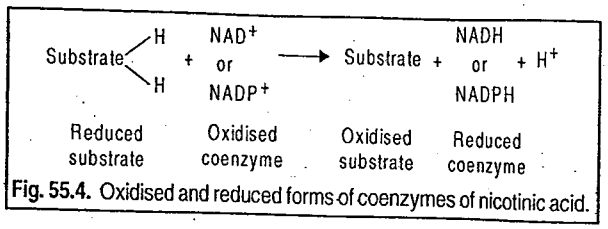


Fig. 55.4. Oxidised and reduced forms of coenzymes of nicotinic acid.

Chemistry and source

Nicotinic acid (Pyridine-3-carboxylic acid or 3-carboxypyridine) is a derivative of pyridine, with a carboxyl group (COOH) at the 3-position. The other form of nicotinic acid is the corresponding amide, nicotinamide (niacinamide), where the carboxyl group has been replaced by a carboxamide group ($CONH_2$). Nicotinic acid functions in body as coenzymes in the forms of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). The nicotinic acid occurs in these two nucleotides in the form of nicotinamide. Plants and most animals can make nicotinic acid from some precursors, particularly the amino acid tryptophan.

Nicotinic acid is obtained from liver, meat, fish, cereals and cereal by-products, nuts and legumes. Corn protein is low in tryptophan; therefore, the nicotinic acid in corn is largely unavailable.

Absorption and fate

Both nicotinic acid and nicotinamide are absorbed readily from all parts of the intestinal tract. After absorption, the vitamin is distributed to all tissues. Little of the vitamin is stored, but liver usually contains appreciable amounts. Nicotinic acid and nicotinamide are metabolised mainly by methylation in the liver and some is excreted in urine as N-methylnicotinamide.

Physiological functions

The pyridine nucleotides NAD and NADP, the active forms of nicotinic acid, function as coenzymes of a large number of oxido-reductases. They act as electron acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom from specific substrate is transported as a hydride ion to nicotinamide portion of the oxidised forms of these coenzymes (symbolised NAD^+ and $NADP^+$) to yield the reduced coenzymes (symbolised NADH and NADPH, respectively); the other hydrogen from the substrate becomes a hydrogen ion (Figure 55.4). The reduced pyridine nucleotides, in turn, are deoxidised by flavoproteins (containing riboflavin). Cells generally delegate NAD to enzymes in catabolic pathways, whereas NADP is utilised in synthetic pathways.

An additional and equally important function of NAD is its role as a substrate in poly- and mono-ribosylation reactions, which are important to many cellular functions. In the nucleus of cell, polyribosylation of histones precedes the normal process of DNA repair.

Disorders of Nicotinic Acid

Deficiency of nicotinic acid

Deficiency of nicotinic acid/niacin (hyponiacinosis) is rare in animals due to the presence of high content of nicotinic acid in most natural animal feeds. However, ration rich in maize (corn, the only grain low in niacin) may lead

to hyponiacinosis in animals, particularly in pigs. Chronic lack of nicotinic acid (vitamin B_3) in the human diet results in pellagra. When a deficiency of nicotinic acid exists, the normal rate of dehydrogenation cannot be maintained; therefore, oxidative delivery of energy from the foodstuffs to the functioning elements of all cells cannot occur at normal rates. Lack of nicotinic acid and therefore deficiency of NAD is thought to be contributing factor to the skin lesions because of the inability of cells to carry out polyribosylation reaction.

Deficiency of nicotinic acid is pigs in characterised by rough scaly skin, alopecia, inappetance, and oral and gastrointestinal ulceration with diarrhoea. Posterior paralysis may also occur. In chicks, perosis, inflammation of tongue and dermatitis may be noted. In dogs, nicotinic acid deficiency is characterised by 'black tongue' or 'brown mouth'. In humans, pellagra is characterised by signs and symptoms related to skin, gastrointestinal tract and central nervous system, a triad frequently referred to as dermatitis, diarrhoea and dementia, or the three Ds.

Daily requirement of nicotinic acid can be met not only by nicotinic acid but also by nicotinamide and amino acid tryptophan. Therefore, nicotinic acid requirement is influenced by the quantity and the quality of dietary proteins. Depending on the species, the conversion of tryptophan to nicotinic acid produces 1 mg of nicotinic acid from every 60 mg of tryptophan degraded. In general, nicotinic acid is needed in amounts corresponding to 10-25 mg/kg of diet. In pigs, 10-20 g per tonnes of feed supplies sufficient nicotinic acid for all ages. In cats, nicotinic acid is usually not synthesised from tryptophan hence nicotinic acid is an obligatory dietary factor.

PHARMACOLOGY OF NICOTINIC ACID

Nicotinic acid and Nicotinamide

Nicotinic acid and nicotinamide are prepared synthetically. Both nicotinic acid and nicotinamide are white crystalline, odourless or almost odourless powders, the nicotinic acid with a slight acid taste and the amide with as intensely bitter taste. The nicotinic acid is less soluble in alcohol, soluble in water (1 : 50) and insoluble is ether, whereas the amide is much more soluble in water (1 : 1) and alcohol but only slightly soluble in ether. Nicotinic acid and nicotinamide are identical in their function as vitamins. However, nicotinamide does not reduce cholesterol or cause flushing as seen with nicotinic acid. Both are absorbed rapidly when given orally, subcutaneously or intramuscularly.

Pharmacological doses of nicotinic acid have occasionally been associated with some side effects such as skin flushing and itching, dry skin and skin rashes including eczema exacerbation. These symptoms are generally related to nicotinic acid's role as the rate limiting cofactor in the histidine decarboxylase enzyme which converts l-histidine into histamine. High-dose nicotinic acid in human has reported to elevate blood sugar, thereby worsening diabetes mellitus. Nicotinic acid at extremely high doses can have life-threatening acute toxic reactions

Nicotinic acid and nicotinamide are used in therapeutic doses for the treatment of hyponiacinosis. In humans, response to nicotinic acid occurs as redness and swelling of tongue disappears and sialorrhoea diminishes within 24 hours. In veterinary medicine, nicotinic acid is often used as a part of multivitamin therapy. It may also be used separately for prophylaxis and treatment of nicotinic acid deficiency in pigs, dogs and cats. Marked overestimation may cause adverse effects and vascular side effects. The nicotinamide should be used preferably, as it provides the vitamin in an immediately utilisable form and eliminates any vascular side effects.

Dose

Nicotinic acid

Dogs & cats	:	5 – 10 mg/kg, IM.
	:	10 – 30 mg/kg, PO.
Pigs	:	100 – 300 mg (total), SC.
	:	200 – 800 mg (total), PO.

Nicotinamide

Dogs & cats	:	500 – 1000 mg (total), IM once, followed by 200 – 300 mg (total), IM at 4 hour intervals, then 200 mg (total), PO, once daily for 2 weeks.

PANTOTHENIC ACID / VITAMIN B_5

Pantothenic acid, also called pantothenate or vitamin B_5, is a water soluble vitamin and an essential growth substance, which is widely distributed in animals and plants. It is probably the least stable of all the B vitamins. Animals require pantothenic acid to synthesize coenzyme-A (CoA), as well as to synthesize and metabolize proteins, carbohydrates and fats.

Chemistry and source

Pantothenic acid consists of pantoic acid linked to β-alanine. Pantothenic acid is transformed in the body to 4'-phosphopantetheine by a series of steps, which is then incorporated into either coenzyme A (CoA, CoA-SH) or acyl carrier

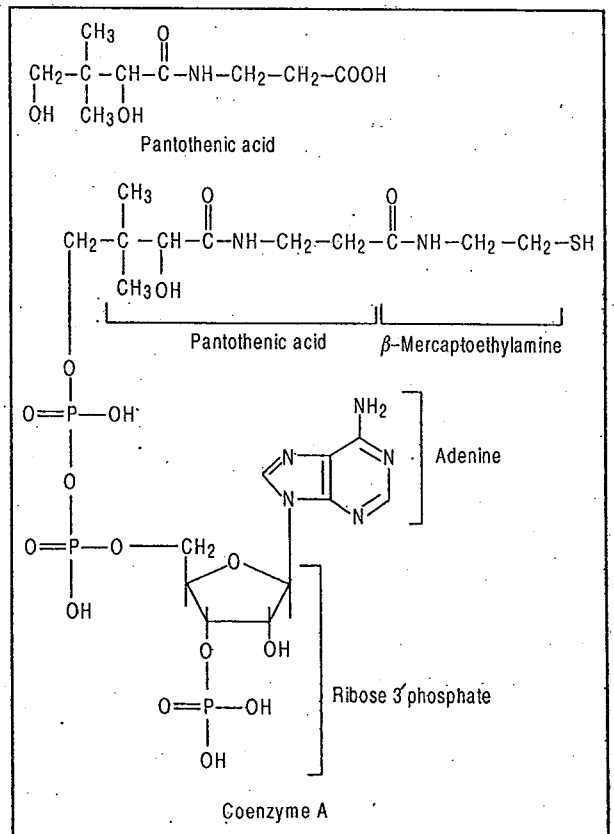


Fig. 55.5. Chemical structures of pantothenic acid and coenzyme A.

protein, the functional forms of the vitamin. Only the dextrorotatory (D) isomer of pantothenic acid possesses biological activity.

Pantothenic acid is ubiquitous. It is particularly abundant in meat, yeast, egg yolk and milk; the richest source being the royal jelly of the honeybee. Milk, peanuts and wheat also contain appreciable quantities of the vitamin. Pantothenic acid is synthesised also in the rumen.

Absorption and fate

Pantothenic acid in food is mostly present in the form of CoA or acyl carrier protein (ACP). Within the lumen of intestine, CoA and ACP are hydrolyzed into 4'-phosphopantetheine, which is then dephosphorylated into pantetheine. Pantetheinase, an intestinal enzyme, hydrolyzes pantetheine into free pantothenic acid. The free pantothenic acid is then absorbed readily from the GI tract via a saturable, sodium-dependent active transport system. In high concentration when the active system becomes saturable, some pantothenic acid is absorbed also by passive diffusion process. After absorption pantothenic acid is distributed to all tissues. Pantothenic acid apparently is not degraded in the body. Urinary output of pantothenic acid is directly proportional to dietary input and has been used as index of adequacy.

Physiological functions

Pantothenic acid is a component of coenzyme A and as such is involved in acetylation processes throughout the body. The functions of coenzyme A are to serve as a carrier of acyl groups in various enzymatic reactions. Such reactions are important in the oxidative metabolism of carbohydrates (e.g. conversion of decarboxylated pyruvic acid into acetyl-CoA before its entry into the TCA cycle), gluconeogenesis, degradation of fatty acids and synthesis of sterols, steroids hormones and porphyrins. Coenzyme A also participates in the post-translational modifications of proteins. Pantothenic acid (as a component of phosphopantothene) also participates in fatty acid synthesis as it is present at the active site of acyl carrier protein (ACP), a component of the fatty acid synthesis complex.

Pantothenic acid in the form of CoA is also required for acylation and acetylation, which are involved in signal transduction and enzyme activation and deactivation, respectively.

Disorders of Pantothenic Acid

Deficiency of pantothenic acid

Deficiency of pantothenic acid in human and animals is not known to occur under natural conditions except in pigs on rations based on corn. The deficiency of pantothenic acid is characterised by impaired acetyl and acyl metabolism hence depressed metabolism of both carbohydrates and fats. Deficiency of pantothenic acid results in dermatitis and loss of hair in all species, particularly in the region of the mouth and eyes. In pigs, anorexia, inefficient food utilisation, ulcerative dermatitis, alopecia, intestinal necrosis, diarrhoea and incoordination with a spastic, goose-stepping gait are characteristic.

Impaired energy production, due to low CoA levels, may cause symptoms of irritability, fatigue and apathy. Deficiency in pantothenic acid can also cause hypoglycaemia or an increased sensitivity to insulin. Acetylcholine synthesis is also impaired; therefore, neurological symptoms such as numbness, paraesthesia and muscle cramps can also appear in deficiency.

Pantothenic acid is a required dietary nutrient in all species other than ruminants, which synthesise it in the rumen. For most animals, the requirements for pantothenic acid are 10-20 mg per kg diet. Dogs should receive about 0.05 mg/kg body wt. of pantothenic acid daily.

Excess of pantothenic acid

Ingestion of large amount of pantothenic acid in diet apparently has no adverse effects on body.

PHARMACOLOGY OF PANTOTHENIC ACID

Pantothenic acid

Pantothenic acid is commercially available as calcium pantothenate. Calcium pantothenate is a white crystalline, hygroscopic and bitter tasting powder that is soluble in water. Calcium pantothenate is often used in dietary supplements because, as a salt, it is more stable than pantothenic acid in the digestive tract, allowing for better absorption. Pantothenic acid has no significant adverse effects, even in large doses.

Calcium pantothenate is used mainly in pigs for treatment and prevention of pantothenic acid deficiency. No clearly defined uses for pantothenic acid exist in other species, although it is often included in multivitamin preparations and in products for oral, topical or parenteral administration.

Dose

Calcium pantothenate

For treatment and prevention

Pigs : 0.5 mg/kg/day, PO.

As feed additive

Pigs : 10 – 12 g/tonne of feed.

Panthenol

Panthenol (Pantothenyl alcohol) is an alcohol analogue of pantothenic acid. It exists as a highly viscous transparent liquid at room temperature that is soluble in water, alcohol and propylene glycol and slightly soluble in glycerine. On ingestion, panthenol is metabolized to pantothenic acid. The panthenol is slightly more stable than the calcium pantothenate and is often used as a source of the vitamin in multivitamin supplements. Panthenol is also a humectant, emollient and moisturizer and is often used in shampoos and hair conditioners. It is sometimes mixed with allantoin in concentrations of up to 2-5%, for the treatment of sunburns, mild burns and minor skin disorders.

PYRIDOXINE / VITAMIN B_6

Pyridoxine is the original vitamin B_6 first identified as essential factor for prevention of dermatitis. Now the term vitamin B_6 is collectively applied to pyridoxine and related natural compounds. pyridoxal and pyridoxamine. All three vitamin B_6 compounds viz. pyridoxine, pyridoxal and pyridoxamine have shown to possess the same biological properties. The vitamin B_6 is one of the most stable of the vitamin B complex.

Chemistry and source

Pyridoxine is 3-hydroxy-4,5-bis-(hydroxymethyl)-2-methylpyridine. Vitamin B_6 compounds differ in nature of the substituent on the carbon atom in position 4 of pyridine nucleus i.e. the primary alcohol is pyridoxine, the corresponding aldehyde is pyridoxal, and an aminoethyl group is pyridoxamine. Each of these compounds can be interconverted and can be utilised by

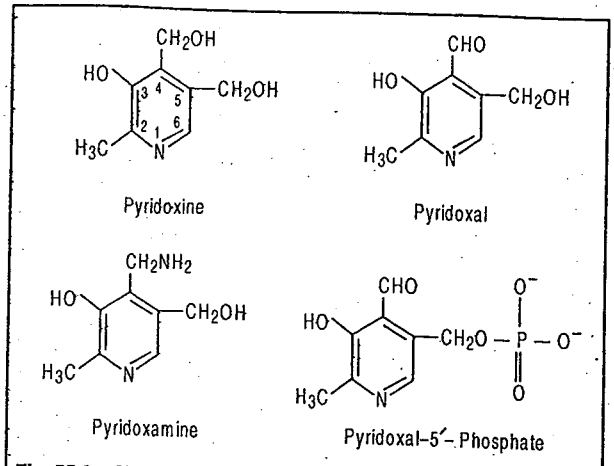


Fig. 55.6. Chemical structures of vitamin B_6 compounds and their active coenzyme form pyridoxal - 5'-phosphate.

mammals after conversion to the active form pyridoxal 5'-phosphate (B_6 PLP).

Pyridoxine, pyridoxal and pyridoxamine exist naturally. Important sources of these include yeast, grains, potatoes, meat, liver, eggs, fish, meat, legumes, soybeans and vegetables. Ruminants and many herbivores meet a substantial part of their vitamin B_6 requirements from intestinal microbial synthesis. Pyridoxine is sensitive to ultraviolet light and oxidation and substantial loses occur during cooking.

Absorption and fate

Pyridoxine, pyridoxal and pyridoxamine are absorbed readily from the GI tract. The absorption of vitamin B_6 largely occurs in the jejunum and ileum via passive diffusion. After absorption, these are converted to pyridoxal-5'-phosphate, the active form of the vitamin. Pyridoxal phosphate accounts for at least 60% of the circulating vitamin B_6. When pyridoxal-5'-phosphate is in excess, it can be converted to pyridoxic acid that is the principal excretory product of vitamin B_6. Like other B vitamins, little storage of vitamin B_6 occurs in body.

Physiological functions

The pyridoxine coenzymes are extremely versatile, functioning in a large number of different enzymatic reactions in which amino acids or amino groups are transferred or transformed. The most common type of enzymatic reactions requiring pyridoxal phosphate as coenzyme is transamination, the transfer of α-amino group of an amino acid to the α-carbon atom of an α-keto acid. As a result, vitamin B_6 plays many key roles in metabolism especially protein synthesis. Important enzymes utilising vitamin B_6 as coenzyme include aminotransferases, decarboxylases, dehydrotases and racemoses.

The pyridoxal phosphate acts as a cofactor for the enzyme aromatic amino acid decarboxylase that converts the precursors 5-hydroxytryptophan (5-HTP) into serotonin and levodopa (L-DOPA) into dopamine, norepinephrine and epinephrine. As such, vitamin B_6 helps in the synthesis of monoamine neurotransmitters serotonin, dopamine and norepinephrine. The pyridoxal phosphate is also required to create physiologically active amines by decarboxylation of amino acids such as histamine from histidine, serotonin from tryptophan and gamma-aminobutyric acid (GABA) from glutamate.

Pyridoxal phosphate aids in the synthesis of haem, by serving as a coenzyme for the enzyme aminolevulinic acid (ALA) synthase. It is also required in the glucose and lipid metabolism. Pyridoxal phosphate has been implicated in increasing or decreasing the expression of certain genes.

Disorders of Vitamin B_6

Deficiency of vitamin B_6/ Hypopyridoxinosis

A deficiency of vitamin B_6 in diet occurs very rarely under natural conditions, as most diets provide adequate amounts of the vitamin. Drug-induced vitamin B_6 deficiency can occur following administration of the antitubercular drug isoniazid or penicillamine used in treatment of Wilson's disease. Linatine (1-amino-D-proline), a naturally occurring antagonist to vitamin B_6 that is present in flax seed, also induces vitamin B_6 deficiency by forming a stable product with pyridoxal phosphate. Antimetabolites to pyridoxine (e.g. 4-deoxypyridoxine) have also been synthesised which block the action of vitamin and produce signs and symptoms of vitamin B_6 deficiency.

Deficiency of vitamin B_6 results in seborrhoeic skin rashes, glossitis and stomatitis. High concentrations may cause peripheral neuropathy and nervous symptoms. In dogs and pigs, microcytic and hypochromic anaemias are seen. Dietary lack of pyridoxine in lower animals can cause acrodynia (a dermatitis characterised by hyperkeratosis and acanthosis of skin), decreased rate of growth, development of fatty liver, anaemia and evidence of mental deterioration. The most important signs of vitamin B_6 deficiency relate to defects in amino acid metabolism. Neurological signs occur as result of reduced synthesis of important biogenic amines from amino acid precursors. Anaemia may occur as a consequence of decreased haem synthesis.

The requirements of vitamin B_6 by animals are possibly related to their intake of protein and amino acids. The daily requirement of pyridoxine in pigs is about 0.1 mg/kg body weight or 1 mg/kg of solid food. Certain strains of chickens have high requirements for pyridoxine.

Excess of vitamin B_6

In animals, there is no evidence for vitamin B_6 toxicity produced by its excessive intake in diets.

PHARMACOLOGY OF PYRIDOXINE

Pyridoxine

Pyridoxine hydrochloride is prepared synthetically and is used commercially for prophylaxis and treatment of vitamin B_6 deficiency. Pyridoxine hydrochloride exists as a white crystalline powder soluble in water but only slightly soluble in alcohol and other organic solvents. Pyridoxine produces negligible pharmacological effects after either oral or intravenous administration. Absorption, metabolism and excretion of synthetic pyridoxine are similar to vitamin B_6 compounds as discussed earlier.

Pyridoxine has been recommended for the treatment of vomiting due to excessive stilbestrol dosage or x-ray irradiations in man. Pyridoxine is considered as an antidote for seizures and acidosis in patients those have ingested an overdosage of isoniazid. Pyridoxine is also used therapeutically in patients receiving isoniazid to prevent the development of peripheral neuritis. Occasionally, pyridoxine is used clinically for the treatment of pyridoxine-responsive anaemias in human patients. In human and veterinary medicines, pyridoxine is more commonly

used as a component of multivitamin preparation. Although, pyridoxine has low acute toxicity, neurotoxicity and symptoms dependency have been reported in human beings after its prolonged use.

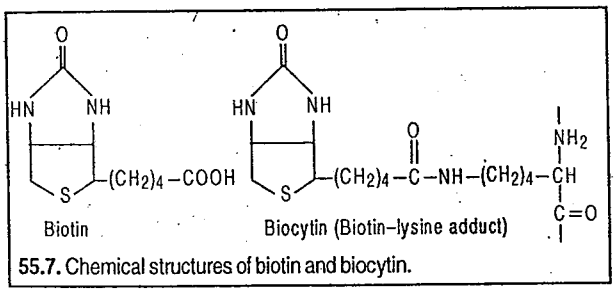
BIOTIN / VITAMIN B_7

Biotin or Vitamin B_7 is a sulphur containing organic acid first isolated from a liver concentrate known to contain growth factor for yeast. This water-soluble vitamin was formerly known by the names of vitamin H, bios IIb, coenzyme R and W factor. Biotin is a coenzyme in the metabolism of fatty acids and leucine, and plays a role in gluconeogenesis.

Chemistry and source

Biotin contains fused imidazole and thiophene rings (Figure 55.7). A valeric acid substituent is attached to one of the carbon atoms of the thiophene ring. In natural materials, biotin exists either in the form of free biotin or in its derivative forms. These derivatives are the biocytin and the D and L sulphoxides of biotin.

Biotin is found in almost all plant and animal materials and is also synthesised by microbes in the GI tract. Liver, egg, yolk, milk, fish, nuts and cereals including soybeans, rice, barley, corn and wheat are rich source of biotin. Biotin is stable to cooking, but is unstable in alkaline solution and in presence of oxidising agents. Avidin, a protein present in raw egg white, binds biotin and prevents its absorption. Other antagonists of biotin include biotin sulphone, desthiobiotin and certain imidazolidone carboxylic acid.



55.7. Chemical structures of biotin and biocytin.

Absorption and fate

Biotin exists in food mainly as protein bound form or biocytin. The protein bound form is required to be broken down by protease prior to absorption. Biotin bioavailability may be variable, depending on the type of food being consumed and the extent of proteolysis being carried out in the GI tract. The biotin present in corn is readily available, but that present in many grains show availability of about a 20-40%. Biotin is excreted mainly unchanged in the urine. Mammals are probably unable to degrade the ring system of biotin. Little of the vitamin is stored in body.

Physiological functions

Biotin functions in enzymatic reactions as a cofactor for CO_2-fixing enzymes. These include acetyl-CoA carboxylase that participates in fatty acid synthesis, propionyl-CoA carboxylase that is essential for odd-chain fatty acid metabolism, and pyruvate carboxylase that in involved in formation of oxaloacetate and in carboxylation of β-methylcrotonyl-CoA. Biotin molecule is combined covalently with these carboxylases by peptidyl linkages between the carboxylic acid moiety of biotin and the ε-amino group of a specific lysine residue of enzyme protein. This biotinylation reaction requires ATP and is catalyzed by holocarboxylase synthetase. The biotin-lysine adduct is called biocytin. Bound biotin or biocytin serves as an intermediate carrier of CO_2 during action of the carboxylating enzymes mentioned above.

When biotin-containing carboxylates are degraded, biocytin is cleaved by the enzyme biocytinase to generate free biotin for reutilisation. In absence of this enzyme, biotin deficiency occurs in newborns.

Disorders of Biotin

Deficiency of biotin

Biotin deficiency occurs rarely under normal conditions in domestic animals. High dosing with egg white, which contains a biotin antagonist avidin, may cause biotin deficiency called egg-white injury in fur bearing animals and pigs, but it is not of much clinical importance.

Biotin deficiency leads to impairment of gluconeogenesis and fat metabolism and may induce severe metabolic acidosis. Alopecia and dermatitis are characteristics of biotin deficiency in most animals and birds. Naturally occurring outbreaks of lameness in gilts and sows associated with lesions of the soles and walls of hooves have been associated with biotin deficient diets.

The biotin requirements of animals are relatively low. For broiler chickens on a high wheat intake, the diet should be supplemented with 75 µg per kg of feed for prophylaxis. The dietary supplementation of 4-5 mg biotin/sow/day is considered adequate.

Excess of biotin

There are no reported cases of adverse effects from receiving high doses of biotin.

PHARMACOLOGY OF BIOTIN

Biotin

Biotin is commercially available as a white crystalline powder, which is soluble in water and alcohol but not in other solvents. Biotin is administered mainly by oral route. Ingested biotin is absorbed rapidly from the GI tract and excreted predominantly as unchanged drug. Except for rare instances of biotin deficiency syndrome, there are no clearly defined therapeutic uses of biotin. Biotin is also present in some multivitamin preparations. Biotin toxicity has not been reported in human beings and animals even at very high doses.

Dose

For biotin deficiency

Dogs & cats : 0.1 mg (total), PO, 2 times daily for 3 days, then 0.05 mg (total), 2 times weekly.

Chickens : 0.1 mg (total), once only (single dose).

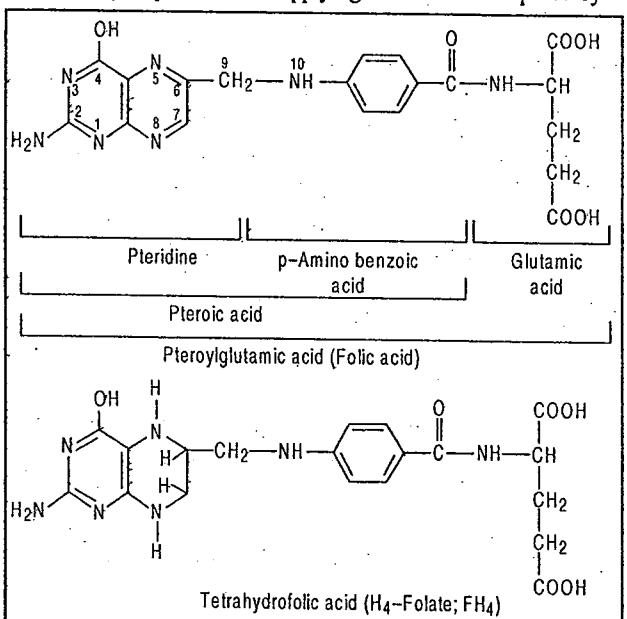
FOLIC ACID / VITAMIN B_9

Folic acid or Pteroylglutamic acid is a water soluble vitamin that was found first in spinach leaves (Latin *folium* = leaf) and is now known to widely distributed in plants. Originally the term was used exclusively for folic acid, but now it is often used collectively for folic acid and its oligoglutamic acid conjugates, called folates. Some synonyms for folic acid are pteroylglutamic acid, pteroylmonoglutamic acid, folacin, liver *Lactobacillus casei* factor, vitamin B_9, vitamin M, vitamin B_c, factor U and factor R. Folic acid along with vitamin B_{12} is essentially required for DNA synthesis and cell proliferations.

Chemistry and source

Chemically folic acid (pteroylglutamic acid) contains three characteristic building blocks – substituted pteridine, para-aminobenzoic acid (PABA) and glutamic acid. It probably does not occur in nature (food) as such, but mainly as the parent compound of a group of similarly acting folate compounds. These compounds are conjugates of folic acid with extra-molecules of glutamic acid attached through peptide linkages giving rise to folate polyglutamates (e.g. vitamin B_c conjugate is a heptaglutamic acid conjugate and 'fermentation *L. casei* factor' is a triglutamic acid conjugate). Folic acid in body is reduced at the 5, 6, 7 and 8 positions to tetrahydrofolic acid (THFA), the active coenzyme form, which acts as an acceptor of a number of one-carbon units such as methyl, hydroxymethyl and formyl. Reduction brings the nitrogens at positions 5 and 10 closer together with changes in their electrochemical properties, which facilitates formation of various THFA single carbon derivatives. The most important forms of the coenzyme which are synthesised by these reactions are listed in Figure 55.8

The natural sources of folic acid include yeast, wheat, green food, meat, liver and some fruits. Intestinal bacteria in all species are probably responsible for supplying some vitamin required by the animals. A dietary source of folic acid is necessary for all species and pastures provide an adequate intake.



	Position	Radical	Congener	Abbreviation (s)
1.	N^5	$-CH_3$	N^5-Methyl tetrahydrofolate	5-CH_3—H_4—Folate; 5-Methyl-FH_4.
2.	N^5	-CHO	N^5-Formyl tetrehydrofolate (Folinic acid)	5-CHO—H_4—Folate; 5-CHO-FH_4.
3.	N^5	-CHNH	N^5-Formiminotetra-hydrofolate	5-CHNH—H_4— Folate; 5-CHNH-FH_4.
4.	N^{10}	$-CH_2OH$	N^{10}-Hydroxymethyl tetrahydrofolate	10-CH_2OH—H_4— Folate; 10-CH_2OH-FH_4.
5.	N^{10}	-CHO	N^{10}-Formyl tetrahydrofolate	10-CHO—H_4—Folate; 10-CHO-FH_4.
6.	N^{5-10}	$-CH_2-$	N^5, N^{10}-Methylene-tetrahydrofolate	5, 10-CH_2—H_4—Folate; 5, 10-CH_2-FH_4.
7.	N^{5-10}	-CH-	N^5, N^{10}-Methenyl-tetrahydrofolate	5, 10—CH—H_4— Folate; 5, 10-CH-FH_4.

Fig. 55.8. Chemical structures and nomenclature of folic acid, tetrahydrofolic acid and congeners.

Absorption and fate

Folic acid is present in food in the form of folate polyglutamates (the naturally occurring forms), which are hydrolysed by the enzyme α-glutamyl transferase within the brush border of intestinal mucosa to form monoglutamate. Most of the folate monoglutamate is reduced by intestinal dihydrofolate reductase and then methylated to methyl tetrahydrofolate (CH_3-H_4-folate) during gastrointestinal transport. Once absorbed, methyl tetrahydrofolate is transported rapidly to tissues in association with certain plasma proteins. Cellular uptake of CH_3-H_4-folate is by active processes. Methyl-tetrahydrofolate is functionally inactive form of folate, but following uptake into cells it acts as a methyl donor for the formation of methylcobalamin (see vitamin B_{12}) and as a source of tetrahydrofolate (H_4-folate) and other folate congeners.

Folate is stored in the liver and cells as polyglutamates. Some folic acid also undergoes enterohepatic cycle. Normally, small amounts of folic acid are excreted in the urine and stool; however, when very high concentration is present, 50-90% of folic acid may be excreted in the urine.

Physiological functions

Folic acid is inactive as such and is reduced in body to the active coenzyme form tetrahydrofolate (H_4-folate) in two-step reactions (Figure 55.9). Tetrahydrofolate carries out a number of one-carbon transfer reactions i.e. it serves as an intermediate carrier of methyl (-CH_3), hydroxymethyl (-CH_2OH), and formyl (-CHO) groups in a large number of enzymatic reactions in which such groups are transferred from one metabolite to another, or are interconverted. In all reactions, polyglutamates are more active than monoglutamates.

a. Folic acid is essentially required for the generation of thymidylates, an essential constituent of DNA. The reaction is catalysed by enzyme thymidylate synthetase in which 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4 folate) donates a methylene group and converts deoxyuridylic acid (deoxyuridylate monophosphate) to deoxythymidylic acid (deoxythymidylate monophosphate) (Figure 55.10). The thymidylate synthetase reaction is rate-limiting step in the mammalian DNA synthesis.

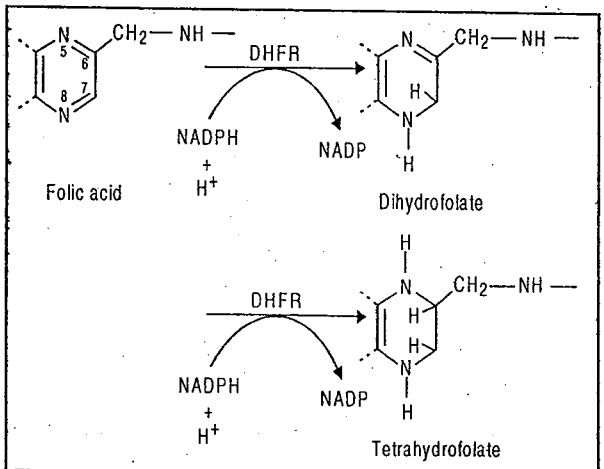


Fig. 55.9. Reduction of folic acid to dihydrofolate and then to tetrahydrofolate by enzyme dihydrofolate reductase (DHFR).

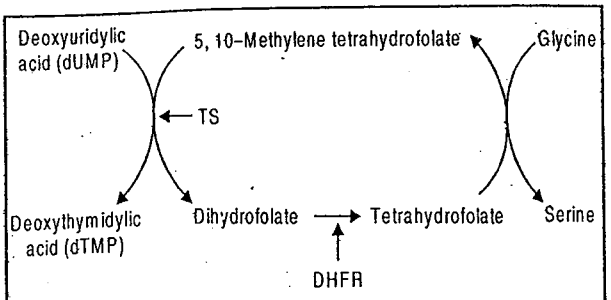


Fig. 55.10. Synthesis of deoxythymidylic acid.
Abbreviations : dUMP = deoxyuridylate monophosphate ; dTMP = deoxythymidylate monophosphate ; TS = thymidylate synthetase ; DHFR = dihydrofolate reductase.

b. Folic acid is required for the conversion of homocysteine to methionine. This reaction requires methyl-tetrahydrofolate (CH_3-H_4-folate) as methyl donor and utilises vitamin B_{12} as a cofactor (see Figure 55.12). This reaction represents an important interaction between two important B vitamins.

c. The *de novo* synthesis of purine nucleotides requires participation of derivatives of folic acid.

d. Folates are also important in various amino acid interconversion reactions and protein synthesis. For example, conversion of serine to glycine requires tetrahydrofolate as an acceptor for a methylene group from serine and utilises pyridoxal phosphate as a cofactor. It results in the formation of 5,10-CH_2-H_4-folate, an essential coenzyme for the synthesis of thymidylate (Figure 55.8). In histidine metabolism, H_4-folate is required for the removal of the formimino group from the formiminoglutamic acid.

Disorders of Folic Acid

Deficiency of folic acid

Deficiency of folic acid (hypofolicosis) is a rare entity in domestic animals under natural conditions. However, folic acid has numerous and complex interrelationship with other nutrients hence possibility of hypofolicosis playing a role in abnormal conditions should not be overlooked. In humans, folate deficiency is a common complication of diseases of small intestine, which interfere with the absorption of folate from food and the recirculation of folate through enterohepatic cycle. Deficiency causes include malabsorption syndromes (e.g. sprue and ileitis), defective folate stores (e.g. liver disease and recurrent renal dialysis), therapeutic use of antifolate compounds (e.g. methotrexate), prolonged cooking of food and increased demands due to pregnancy and lactation.

Like vitamin B_{12}, deficiency of folic acid produces clinical signs of macrocytic anaemia and dissynchronies in growth and development. This occurs mainly due to interference with purine and DNA synthesis. The appearance of megaloblastic anaemia after deprivation of folate in human beings appears much more rapidly than that caused by vitamin B_{12} deficiency because storage of folate is limited *in vivo*.

In general, the requirements for folic acid range from 1 to 10 mg per kg diet for most animals. Requirement of folic acid increases under certain conditions such as when natural or pharmacological folic acid antagonists (e.g. methotrexate) are present in the diet.

Excess of folic acid

The risk of toxicity from folic acid is low, because folate is a water-soluble vitamin and is regularly removed from the body through urine.

PHARMACOLOGY OF FOLIC ACID

Folic acid

Folic acid is a yellow or orange-yellow, odourless crystalline powder. Folic acid is practically insoluble in water and organic solvents, but is soluble in acid or alkaline solution. Its sodium salt is freely water soluble hence the vitamin is available for injection as sodium folate. Folic acid is available commercially as oral preparations (alone or combined product) and as aqueous solution for injection. Absorption of folic acid is fairly well from the intestine and follows the processes as discussed under dietary folic acid.

Indications for folic acid therapy are deficiency of vitamin due to malabsorption, liver diseases, chronic debilitating disease or prolonged therapy with methotrexate, sulphonamides and some anticonvulsants such as phenytoin. In human beings, it is also used prophylactically in subjects at risk of developing folate deficiency such as premature infants, pregnant women, and severe haemolytic anaemias including sickle cell anaemia. Folic acid is commonly given orally. In malabsorption or in severely ill patients, IM injection may be used. No apparent significant adverse effect associated with oral folic acid therapy has been reported. Injections rarely cause sensitivity reactions.

Dose

Dogs : 5 mg (total), PO, once daily.
Cats : 2.5 mg (total), PO, once daily.

Folinic acid

Folinic acid (Leucovorin, citrovorum factor, 5-CHO-H_4-folate) is a 5-formyl derivative of tetrahydrofolic acid. In the body, it is readily converted to tetrahydrofolate and other reduced folic acid derivatives, without requiring the action of dihydrofolate reductase. Accordingly its function as a vitamin is unaffected by inhibition of the dihydrofolate reductase enzyme by drugs such as methotrexate. Folinic acid has vitamin activity almost equivalent to that of folic acid; therefore, it allows for some purine/pyrimidine synthesis to occur in the presence of dihydrofolate reductase inhibition.

Folinic acid is largely used to prevent adverse effects of methotrexate, a dihydrofolate reductase inhibitor (antimetabolites), where it may "rescue" bone marrow and gastrointestinal mucosa cells from methotrexate. Folinic acid is also used in combination with the antineoplastic agent 5-fluorouracil in treating colon cancer. In this case, folinic acid is not acting as a rescue agent, but potentiates the effect of 5-fluorouracil by inhibiting thymidylate synthase. Folinic acid is also sometimes used to prevent toxic effects of high doses of antimicrobial dihydrofolate reductase inhibitors such as trimethoprim and pyrimethamine. Folinic acid is less/not indicated for folic acid deficiency like pernicious anaemia or other megaloblastic anaemias secondary to a deficiency of vitamin B_{12}.

Dose

Dogs & cats : 1 mg/kg, PO, once daily.

CYANOCOBALAMIN / VITAMIN B_{12}

Cyanocobalamin (Cobalamin) or vitamin B_{12} is a complex cobalt-containing water soluble compound. Originally the term vitamin B_{12} was applied only to cyanocobalamin, but now it is used as generic name for cyanocobalamin and some other compounds exhibiting biological activity of cyanocobalamin. But still for most practical purposes, the terms vitamin B_{12} and cyanocobalamin are used interchangeably. Some less commonly used names for cyanocobalamin are cobalamin, maturation factor, erythrocyte maturation factor, antipernicious anaemia factor, animal protein factor and extrinsic factor.

Chemistry and source

Vitamin B_{12} is the most chemically complex of all the vitamins. Vitamin B_{12} compounds are made up of a porphyrin like ring with a central cobalt atom attach to a nucleotide; the coordination bonds are similar to those of iron in the haemoglobin molecule. Various ligands may be covalently bound to cobalt atom forming different cobalamins including cyanocobalamin (vitamin B_{12}), hydroxocobalamin (vitamin B_{12a}), aquocobalamin (vitamin B_{12b}), nitritocobalamin (vitamin B_{12c}), etc. (Figure 55.11). In cyanocobalamin, cyanide occupies one of the coordination positions of the cobalt atom. The active coenzyme forms of vitamin B_{12} generated in the body are methyl-cobalamin (methyl B_{12}) and 5'-deoxyadenosyl-cobalamin (ado-B_{12}, DAB_{12}).

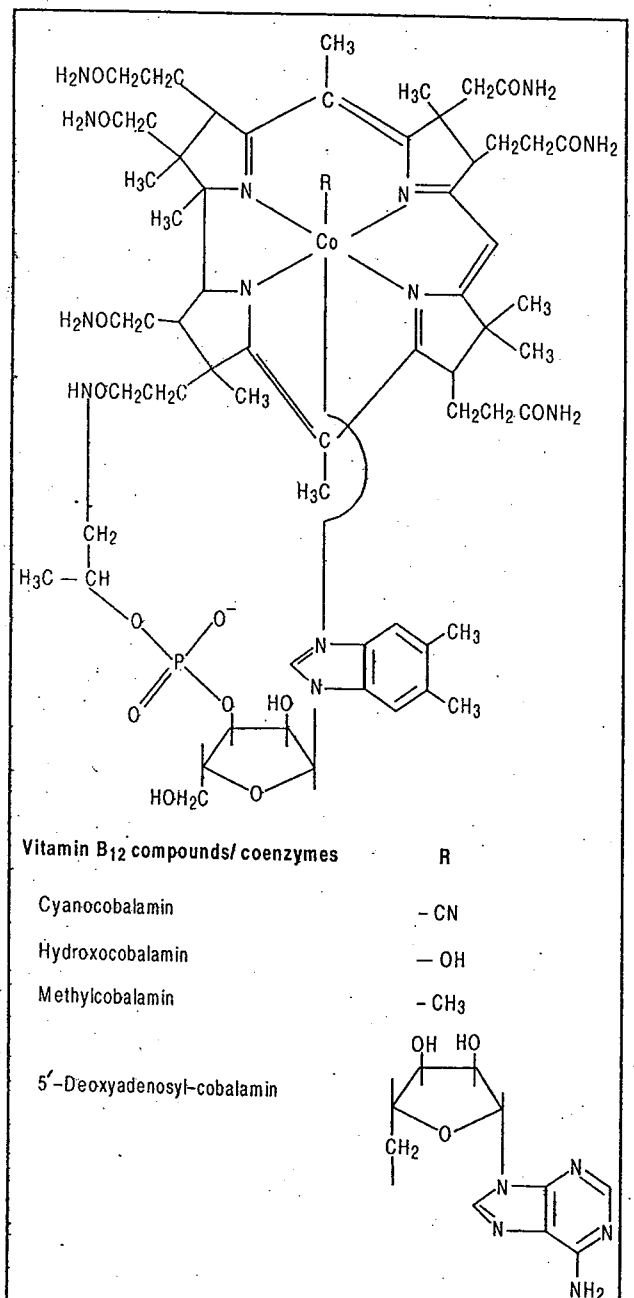


Fig. 55.11. Chemical structures of vitamin B_{12} compounds and their active coenzyme forms.

Cyanocobalamin is very hygroscopic in the anhydrous form and sparingly soluble in water (1:80). It is stable to autoclaving for short periods at 121 °C. Cyanocobalamin is the most famous member of the vitamin B_{12} family, because it is the most air-stable form of vitamin B_{12}.

Vitamin B_{12} is not synthesised by animals or plants. It is synthesised in nature only by microorganisms, and plants and animals acquire the vitamin from microorganisms. In the diet, the primary sources of vitamin B_{12} are meat (particularly liver), fish, milk, eggs and cheese. The only vegetable source is legumes (pulses), which get it from microorganisms harboured in their root nodules. Little vitamin B_{12} is present in green food, fruit or yeast. Microbial synthesis of vitamin B_{12} occurs in the rumen in the presence of adequate cobalt, and in the intestine of other herbivora such as horses. In most species, it is essential as a supplement, if the diet contains no animal protein.

Absorption, transport and fate

Absorption and transport: Vitamin B_{12} is present in food as protein conjugate. Gastric acid and pancreatic enzymes release free vitamin B_{12} in stomach and duodenum (release may also occur by cooking). To avoid gets digested in the GI tract, vitamin B_{12} binds to intrinsic factor (a glycoprotein of 45,000 Mol. wt.) and factor R, which are secreted by the parietal cells (also by pancreas and salivary gland in some species). The vitamin B_{12}-intrinsic factor complex is then carried to the ileum, where the complex interacts with a specific receptor on ileal mucosal cells. Vitamin B_{12} enters the intestinal cell by pinocytosis and then enters blood.

Once in blood, vitamin B_{12} binds with one of at least three specific transport proteins called transcobalamins I, II and III. The transcobalamins transport vitamin B_{12} to cells where it is again transferred into target cells by endocytotic mechanisms. Excess vitamin B_{12} is stored in the liver as the active coenzyme and is slowly released as needed. The supply of vitamin B_{12} available for tissues is directly related to size of the hepatic storage pool and amount of vitamin bound to transcobalamin.

Fate: Vitamin B_{12} is not degraded in the body. It is excreted into the bile but undergoes enterohepatic cycling. This entero-hepatic cycle is important since interference with reabsorption by intestinal disease can result in a continuous depletion of hepatic stores of the vitamin. Excess amount administered is also excreted in urine because it remains free without being bound to transcobalamin.

Physiological functions

Vitamin B_{12} performs several metabolic functions and is required essentially for cell growth and replication. It is normally involved in the metabolism of every cell of the body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production.

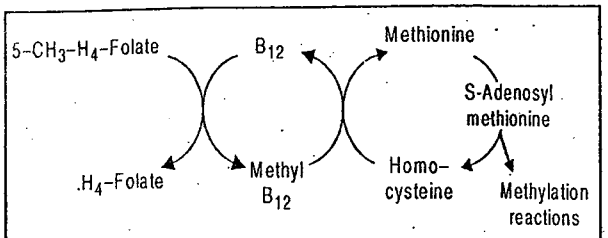


Fig. 55.12. Schematic representation of reactions involving vitamin B_{12} in the synthesis of tetrahydrofolate and methionine.
Abbreviations : 5-CH_3-H_4-folate = 5-methyltetrahydrofolate; H_4-folate = tetrahydrofolate; B_{12} = vitamin B_{12} or cyanocobalamin; methyl B_{12} = methyl cyanocobalamain.

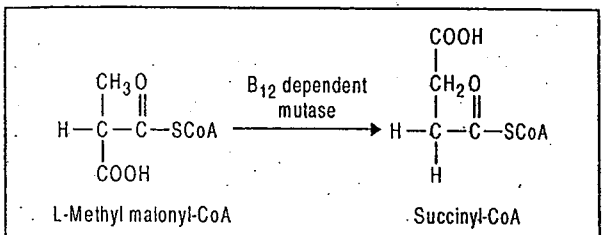


Fig. 55.13. A vitamin B_{12}-dependent mutase reaction.

Methylcobalamin, the active form of vitamin B_{12}, is required for conversion of 5-methyl tetrahydrofolate (methyl FH_4, 5-CH_3-H_4-folate) to tetrahydrofolate (H_4 folate), and homocysteine to methionine (Figure 55.12). Tetrahydrofolate is an active coenzyme form of folic acid (see also folic acid), whereas methionine is needed as methyl donor (in the form of S-adenosyl methionine) in many metabolic reactions. In vitamin B_{12} deficiency, folate remains in the inactive methyl tetrahydrofolate (5-CH_3-H_4-folate) form, which results in depletion of all intracellular folate glutaminate coenzymes. This may result in interruption of a number of one carbon-transfer reactions (see for detail, Folic Acid). The haematological abnormalities those are observed in vitamin B_{12} deficiency are the result of this process that causes defective DNA synthesis. Apart from haematopoietic, other rapidly proliferating tissues also suffer.

The active coenzyme 5-deoxyadenosylcobalamin (ado-B_{12}) is required for isomerisation of methyl-malonyl-CoA to succinyl CoA, as vitamin B_{12} is the coenzyme of methyl-malonyl-CoA mutase. The isomerisation reaction (Figure 55.13) is part of a route by which propionate is converted to succinate. Through this pathway, cholesterol, odd chain fatty acids, some amino acids and thiamine may be used for energy production. The reaction is of particular significance to some herbivores and ruminants, which depend heavily on propionate. In vitamin B_{12} deficiency, the isomerisation reaction cannot occur and the methyl-malonyl-CoA accumulates that results in faulty fatty acids synthesis in neural tissue leading to the neuropathy.

Disorders of Vitamin B_{12}

Deficiency of vitamin B_{12}

Vitamin B_{12} deficiency (Hypocyanocobalaminosis) in ruminants is not seen under natural conditions except when inadequate cobalt in present in the diet. In carnivores, vitamin B_{12} deficiency may occur as a result of inadequate absorption of the vitamin from GI tract or increased body requirements. A number of intestinal diseases or defects can interfere with the absorption of vitamin B_{12}. These include a combination of gastric achlorhydria and decreased secretion of intrinsic factor, pancreatic insufficiency, damaged intestinal mucosa, bacterial overgrowth and certain intestinal parasites. A deficiency syndrome has been reported in young calves on a synthetic ration.

Deficiency of vitamin B_{12} produces clinical signs of anorexia, cessation of growth, loss of condition, incoordination, macrocytic anaemia and muscular weakness. Chronic deficiencies of vitamin B_{12} can promote fatty liver disease and may produce poor reproductive performance. Prolonged vitamin B_{12} deficiency in humans results in serious neurological disorders such as degeneration of the myelin sheath. Paraesthesia, depressed stretch reflexes, poor memory, mood changes and hallucinations have been reported in human patients.

Although microbial synthesis of vitamin B_{12} occurs in rumen and also in intestine of some herbivora, vitamin B_{12} is probably a dietary essential in the young calves, pigs and dogs. The requirements for vitamin B_{12} for most animals are in the range of 2 – 20 µg/kg diet.

Excess of vitamin B_{12}

Vitamin B_{12} has extremely low toxicity and even taking it in enormous doses appears not to be harmful to healthy animals.

PHARMACOLOGY OF VITAMIN B_{12}

Cyanocobalamin and hydroxocobalamin are two principal vitamin B_{12} compounds available for clinical use.

Cyanocobalamin

Cyanocobalamin is a dark red, crystalline, hygroscopic powder, which is soluble in water (aqueous solution has a characteristic red-colour) and alcohol. It is available commercially in pure form or in combination with other vitamins and minerals for injection or oral administration. The commercial source of cyanocobalamin is *Streptomyces griseus*, as a by-product of streptomycin industry. Cyanocobalamin is relatively stable, but alkalis, strong acids, reducing and oxidising agents, and heavy metals cause its decomposition. Cyanocobalamin is absorbed rapidly following parenteral administration. Absorption from the GI tract after oral administration is affected by several factors as discussed earlier.

There are no significant toxicities associated with cyanocobalamin therapy though rare reports of transitory exanthema and anaphylaxis following injection in human patients have been documented. The oral use of cyanocobalamin has occasionally been associated with allergic reactions, dyspnoea and swelling of the face, lips, tongue or throat. Less-serious side effects may include nausea, gastric upset, diarrhoea, joint pain and itching. Cyanocobalamin injection is safe when given by the IM or deep SC route, but it should not be given IV. Vitamin B_{12} shows interaction with a large number of drugs. Antacids and acid-blocking drugs (especially proton-pump inhibitors) may inhibit absorption of B_{12} by decreasing acid concentration in stomach. Aminosalicylic acid can reduce oral vitamin B_{12} absorption, possibly by a general malabsorption syndrome. Nitrous oxide inactivates the cobalamin form of vitamin B_{12} by oxidation. Anticonvulsant drugs such as

phenytoin, phenobarbital and primidone have been associated with reduced vitamin B_{12} absorption.

Cyanocobalamin is indicated primarily for the treatment of vitamin B_{12} deficiency related conditions such as pernicious anaemia. In humans, it is usually prescribed after surgical removal of part or all of the stomach or intestine to ensure there are adequate levels of vitamin B_{12} in the bloodstream. In vitamin B_{12} deficiency, cyanocobalamin is used mainly by parenteral route because vitamin B_{12} deficiency usually occurs due to malabsorption syndrome. Vitamin B_{12} should be given prophylactically only when there is a reasonable probability that a deficiency exists. It is occasionally used as a feed additive for fattening of pigs.

Dose

Dogs	:	0.1 – 0.2 mg (total), PO, once daily.
Cats	:	0.05 – 0.1 mg (total), once daily.
Dogs & cats	:	0.25 – 0.5 mg (total), IM or SC, 1 to 2 times weekly.
Sheep & pigs	:	0.25 – 0.75 mg (total), IM or SC, 1 to 2 times weekly.
Cattle & horses:		1 – 3 mg (total), IM or SC, 1 to 2 times weekly.

Hydroxocobalamin

Hydroxocobalamin is another commercially available vitamin B_{12} form. It is available mainly as a parenteral preparation in highly protein bound form. Hydroxocobalamin is thought to be converted to the active enzymic forms of B_{12} more easily than cyanocobalamin. It has also longer retention times in the body. Hydroxocobalamin is used for treatment of conditions related to vitamin B_{12} deficiency, especially in situations where a sustained effect is desired. Hydroxocobalamin also has an avid affinity for cyanide ion and has been used as an antidote to cyanide poisoning.

Hydroxocobalamin is supplied typically in water solution for injection. Hydroxocobalamin when given IM has been reported to provide more sustained effect being maintaining plasma vitamin B_{12} concentrations in the normal range for up to 3 months. However, administration of hydroxocobalamin is reported to produce formation of antibodies to the transcobalamin-vitamin B_{12} complex.

CHOLINE

Choline (Trimethylethanolamine) is a member of the vitamin B complex, which plays a key role in methyl group metabolism and lipid transport. Choline is a component of lecithin phospholipids, which are found in high concentrations in nervous tissues. As a part of acetylcholine, it is essential for synaptic transmission. Choline is found in egg yolk, liver, yeast, wheat and soybeans, mostly as lecithin. It can also be synthesised *de novo* from ethanolamine, when methionine, dimethylcysteine or betaine is in adequate supply.

Choline deficient animals reflect multiple deficits including fatty liver, cirrhosis, motor incoordination, haemorrhagic renal lesions, and increased incidence of hepatocellular carcinomas, a unique example of nutritional deficiency resulting in neoplasm. Choline deficiency in animals severely compromises renal function. Choline low diets can also cause infertility, growth impairment, bone abnormalities and hypertension.

Supplementary choline is available mainly as phosphatidyl choline and choline chloride. Phosphatidyl choline is used as a supplement in pill or powder form, whereas choline chloride comes as a liquid due to its hydrophilic properties. Choline chloride is sometimes preferred as a supplement because phosphatidyl choline can have gastrointestinal side effects. Choline is a dietary essential for pigs and young calves. For pigs, 1 kg per tonne of food is considered to supply sufficient choline. Choline has also been used in the treatment of milk fever and acetonaemia, where it is reported to improve fatty liver caused by such conditions.

Dose

Dogs	:	44 mg/kg, PO, daily.
Cattle	:	25 – 50 g (total), PO or SC.

INOSITOL

Inositol (Hexahydroxycyclohexane) or mouse antialopecia factor is a member of the vitamin B complex, which is synthesised from glucose-6-phosphate after cyclisation. Inositol is present in the form of phosphatidyl inositol in the phospholipids of cell membranes and plasma lipoproteins and plays important roles in signal transduction and phospholipid assembly. It also functions as an intracellular second messenger in the form of inositol-1,4,5-triphosphate (see for detail Chapter 6). Inositol is found in all tissues of animal or plant origin. It occurs in a number of stereoisomeric forms, the most abundant naturally occurring one is myo-inositol.

Deficiency of inositol in the diet causes alopecia and dermatitis in mice and 'spectacle eyes' in rats. Like choline, a dietary deficiency may result in fatty liver. Clinical deficiencies of inositol have not been recognised in domestic animals because of wide spread distribution of the compound.

CARNITINE

Carnitine (β-Hydroxy-γ-trimethylammonium butyrate) or β-factor plays a major role in the oxidation of long-chain fatty acids. It also facilitates aerobic metabolism of carbohydrates, enhances oxidative phosphorylation and promotes excretion of certain organic acids. Carnitine comes from both the diet and biosynthesis *in vivo*. The primary sources of dietary carnitine are meat and dairy products. In animals, carnitine is biosynthesized primarily in the liver and kidneys from the amino acids lysine (via trimethyllysine) or methionine. Vitamin C (ascorbic acid) is essential to the synthesis of carnitine.

The carnitine exerts a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level. Carnitine deficiency mainly affects lipid metabolism resulting in storages of fat in muscle and functional abnormalities of cardiac and skeletal muscles. An inherited carnitine deficiency has been recognised in some breeds of dogs. Renal tubular disorders causing excessive carnitine excretion may also cause secondary carnitine deficiency.

Carnitine has important therapeutic effects. In human medicine, carnitine is primarily used for heart-related conditions such as angina. Carnitine is occasionally used to improve male fertility by improving sperm quality.

PARA-AMINOBENZOIC ACID

Para-aminobenzoic acid (PABA), sometimes called vitamin B_{10}, is a factor in the vitamin B-complex. It is widely distributed in animal and plant tissues and is found in the highest concentration in liver and yeast. It is an essential growth factor for many bacteria and is also required for chickens. Para-aminobenzoic acid is a part of the folic acid and is required essentially for its formation. It also acts as a coenzyme in the metabolism and utilization of protein. The potassium salt of PABA is used as a drug against certain fibrotic skin disorders. In human medicine, PABA is also occasionally used in pill form in the management of irritable bowel syndrome associated gastrointestinal symptoms. The importance of PABA in veterinary and human medicines also lies due to its antagonism of the sulphonamides (see chapter 59). Dosage and nutritional requirements of PABA in veterinary species are not clear.

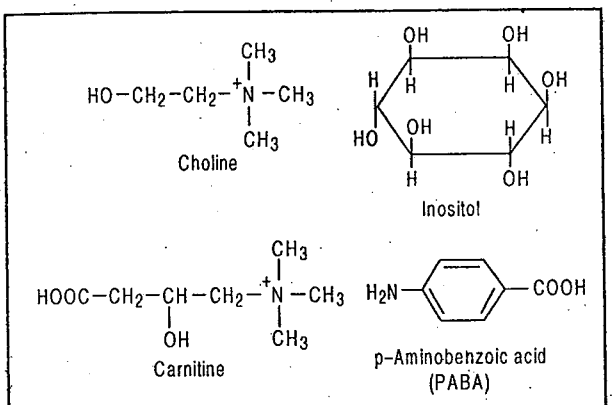


Fig. 55.14. Chemical structures of some B-vitamins.

ASCORBIC ACID / VITAMIN C

Ascorbic acid or Vitamin C is a six-carbon organic acid structurally related to glucose and other hexoses. Ascorbic acid is synthesised by all animals except primates and guinea pigs hence it is not an important dietary essential in any of the domestic animals. In animals, a deficiency of gulonolactone oxidase, the last step in ascorbic acid synthesis, results in the need for a dietary source.

Chemistry and source

Ascorbic acid is a six-carbon ketolactone, which is synthesised from glucose in the liver or kidneys. The glucose needed to produce ascorbic acid in the liver is extracted from glycogen. Ascorbic acid is reversibly oxidised in the body to dehydroascorbic acid, which possesses biological activity similar to that of ascorbic acid. Dehydroascorbic acid is less hydrophilic hence it can move easily across cell membranes.

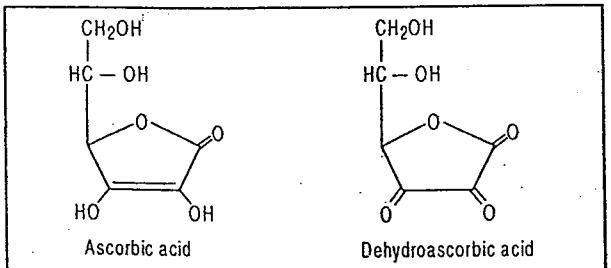


Fig. 55.15. Chemical structures of ascorbic acid and dehydroascorbic acid.

Ascorbic acid is obtained from citrus fruits, tomatoes, potato, cabbage and other vegetables. Domestic animals, however, do not require dietary vitamin C because they can synthesise it *in vivo*. Ascorbic acid is not present in microorganisms.

Absorption and fate

Dietary ascorbic acid is destroyed in the rumen, but is well absorbed from the intestine of simple stomach animals by an energy dependent process. Sodium-dependent active transporters namely sodium-ascorbate co-transporter (SVCTs) and hexose transporter (GLUT) are the two carriers required for absorption. The SVCTs transport the reduced form of ascorbate, whereas GLUT transfer only dehydroascorbic acid form of Vitamin C. Although dehydroascorbic acid is absorbed in higher rate than ascorbate, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to ascorbate. In tissues, highest concentrations of ascorbic acid are found in adrenal and pituitary glands followed by liver, thymus, brain and pancreas. Cellular uptake of ascorbic acid occurs by both active and simple diffusion processes. In human beings, it is partly oxidised to active dehydroascorbic acid and inactive oxalic acid metabolites. The catabolic enzymes which decarboxylate and cleave ascorbic acid are usually induced by excess of ascorbic acid in cells.

Physiological functions

Ascorbic acid performs numerous physiological functions in the body. Ascorbic acid is the most powerful reducing agent available to cells and is of general importance as an antioxidant because of its high reducing potential. It serves as an electron donor (reducing agent) for enzymatic reactions, especially hydroxylation and amidation. Ascorbic acid is particularly important for collagen synthesis and conversion of lysine to carnitine and folic acid to folinic acid. Ascorbic acid promotes the activity of an amidating enzyme thought to be involved in the processing of certain peptide hormones such as oxytocin, vasopressin and cholecystokinin. Ascorbic acid also helps in the conversion of ferric iron to ferrous iron assisting iron absorption from GI tract (see for detail Chapter 36).

Vitamin C is found in high concentrations in immune cells and is consumed quickly during infections. The function of vitamin C in immune system in now clearly known, but its role in some other processes such as in modulation of phagocytosis and production of cytokines and lymphocytes has only been hypothesised. Vitamin C is also called natural anti-histamine as it prevents histamine release and increases its detoxification.

Disorders of Ascorbic Acid

Deficiency of Ascorbic acid

Deficiency of vitamin C leads to scurvy in human beings. Symptoms of scurvy originate primarily from impaired collagen synthesis and may include increased capillary fragility, swollen and bleeding gums, petechial and subperiosteal haemorrhages, deformed teeth, delayed wound healing, impaired bone formation, anaemia and growth retardation. The human body can store only a certain amount of vitamin C, and so the body stores are depleted if fresh supplies are not consumed. In animals, a dermatosis of young calves has been associated with low levels of ascorbic acid in the plasma.

Requirements for ascorbic acid, when needed, in the diet range from 50 to 250 mg per kg diet.

Table 55.1: Important characteristics of vitamins.

Vitamin	Year of discovery	Important Coenzyme or Active Form	Main Function(s)	Deficiency diseases/Important symptoms
Fat Soluble Vitamin				
1. Vitamin A (Retinol)	1913	11-Cis-Retinal	Visual cycle.	Night-blindness, hyperkeratosis and keratomalacia.
2. Vitamin D (Calciferol)	1920	1,25-Dihydroxy-cholecalciferol	Calcium and phosphate metabolisms.	Rickets and osteomalacia.
3. Vitamin E (Tocopherol)	1922		Antioxidant.	White muscle disease (cattle and sheep), encephalomalcia (chicks), yellow fat disease (pigs) and equine degenerative myeloencephalopathy (horses).
4. Vitamin K (Phytomenadione)	1929		Blood clotting factors biosynthesis.	Bleeding diathesis.
Water Soluble Vitamins				
1. Vitamin B Complex				
a. Vitamin B_1 (Thiamine)	1910	Thiamine pyrophosphate (TPP)	Aldehyde group transfer.	Polioencephalomalacia; Beriberi (humans).
b. Vitamin B_2 (Riboflavin)	1920	Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)	Hydrogen atom (electron) transfer.	Ariboflavinosis; Anaemia, curled toe paralysis (chicks).
c. Vitamin B_3 (Nicotinic acid)	1936	Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen atom (electron) transfer.	Hyponiacinosis; Alopecia, posterior paralysis (pigs), black tongue (dogs) and pellagra (humans).
d. Vitamin B_5 (Pantothenic acid)	1931	Coenzyme A (CoA; CoA-SH)	Acyl group transfer.	Alopecia, dermatitis, enteritis and incoordination.
e. Vitamin B_6 (Pyridoxine)	1934	Pyridoxal phosphate	Amino group transfer.	Hypopyridoxinosis; Anaemia, acrodynia and peripheral neuropathy.
f. Vitamin B_7 (Biotin)	1931	Biocytin	Carboxyl transfer.	Alopecia, dermatitis and lameness (Sows).
g. Vitamin B_9 (Folic acid)	1941	Tetrahydrofolic acid (H_4-folate)	One-carbon group transfer.	Hypofolicosis; Megaloblastic anaemia, abnormal growth and development.
h. Vitamin B_{12} (Cyanocobalamin)	1926	Methylcobalamin (methyl B_{12}) and 5'-Deoxyadenosyl-cobalamin (ado-B_{12})	1,2 shift of hydrogen atoms.	Hypocyanocobalaminosis; Megaloblastic anaemia, muscular weakness and paraesthesias.
i. Choline			Role in methyl group metabolism and lipid transport.	Motor incoordination and haemorrhagic renal lesions.
j. Inositol			Role in signal transduction and phospholipid assembly.	Alopecia and dermatitis.
k. Carnitine			Role in oxidation of long-chain fatty acids.	Functional abnormalities of cardiac and skeletal muscles.
l. Para-aminobenzoic acid			Required in formation of folic acid.	Gastrointestinal disorders.
2. Vitamin C (Ascorbic acid)	1920		Co-factor in hydroxylation.	Scurvy (Humans).

Excess of ascorbic acid

Disorders associated with consumption of large doses of vitamin C is not observed in animals. In humans, relatively large doses of ascorbic acid may cause indigestion, particularly when taken on an empty stomach.

PHARMACOLOGY OF ASCORBIC ACID

Ascorbic acid

Ascorbic acid is available commercially as a white crystalline powder without odour but with a sour taste. It is soluble in water, less soluble is alcohol and insoluble is other organic solvents. Commercial ascorbic acid is generally prepared synthetically. Ascorbic acid powder is fairly stable, but slowly darkens on exposure to light. Ascorbic acid solution is rapidly destroyed by oxidations.

Adverse effects due to ascorbic acid have not been reported but overdosage in human beings may induce indigestion, diarrhoea, perspirations, nervous tension and lowered pulse. Nausea, vomiting, flushing of face, headache, fatigue and disturbed sleep have also been reported. The risk of urinary oxalate stones may also be increased.

The clinical indications for use of ascorbic acid has not been clearly determined in domestic animals, though the vitamin is used as adjunct in the treatment of paracetamol and copper poisoning, and for urine acidification (see also Chapter 41). The reducing properties of ascorbic acid have been employed to treat nitrite toxicity induced methaemoglobinaemia, although it is less effective than methylene blue (see Chapter 76). Ascorbic acid has also been used in the infertility in cattle. In human medicine, ascorbic acid is used mainly for the treatment of scurvy and also for prevention of ascorbic acid deficiency in individuals at risk. Vitamin C is available in tablets, capsules, drink mix packets, multi-vitamin formulations, multiple antioxidant formulations, and crystalline powder.

Dose

Dogs : 100 – 500 mg (total), PO, once daily.

Cats : 100 mg (total), PO, 1 to 3 times daily.

For paracetamol toxicity

Cats : 30 mg/kg, PO or SC, 4 times daily for up to 2 days.

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SECTION-XI

DRUGS ACTING ON IMMUNE SYSTEM

CHAPTER 56 GENERAL CONSIDERATIONS

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- Adaptive immunity

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The immune system is a biological defence system of special cells, proteins, tissues and organs which protects an organism against diseases and microorganisms. The presence of a normally functional immune system is extremely important for sustenance of a healthy life. Disorders in the immune system can result in several diseases, including autoimmune diseases, inflammatory diseases and cancer. Immunodeficiency can result in recurring and life-threatening infections such as the acquired immune deficiency syndrome (AIDS), whereas a hyperactive immune system can result in autoimmune diseases like rheumatoid arthritis. Pathogenesis and clinical cure of many diseases and the beneficial and adverse reactions of many drugs are being explained today on the basis of immune mechanisms. Keeping these points in mind, a brief review of the immune system is given is the present chapter.

Immune Defences/Immunity

Immunity may be defined as the state of resistance to invading organisms or toxins which tend to damage body tissues and organs. Immunity has been evolved as a highly sophisticated defence mechanism of higher organisms. In animals, immunity has been broadly classified into two main types – innate immunity and adaptive immunity.

Innate Immunity

Innate or natural immunity is primitive and non-specific immunity that does not require priming. It mainly results from general processes and may include barriers provided by the integument and GI tract, phagocytosis by white blood cells and cells of tissue macrophage system, and antimicrobial actions of many body secretions and chemical compounds such as gastric acid, digestive enzymes and lysosomes. The major effectors of the innate immunity are complement, granulocytes, monocytes/macrophages, natural killer cells, mast cells, basophils, etc. Innate immunity, although very useful, cannot be entirely effective in all cases because many invading organisms overcome this type of immunity. The innate immune system is the dominant system of host defence in most organisms.

Adaptive immunity

Adaptive or acquired immunity is extremely powerful specific immunity that requires antigen exposure or priming. It is caused by a special immune system that forms antibodies and activated lymphocytes, which attack and destroy the specific organisms or toxins. The major effectors of adaptive immunity are B and T cells those mediate cellular and humoral/serological immunity, respectively. The B cells make antibodies which destroy foreign invaders those enter the body openly and grow in the extracellular fluid. The T cells which function as helper, cytolytic and suppressor cells destroy the invaders those grow inside cells where antibodies cannot reach. These cells are important in the normal immune response to infections and tumours and also they mediate transport rejection and autoimmunity. Some of the non-specific

components are also part of adaptive immunity including phagocytic circulating leucocytes and tissue macrophages, leucocyte substances such as lysozymes and soluble components like complements. The components of adaptive immunity are most important and are the primary targets of pharmacological manipulation

COMPONENTS OF IMMUNE SYSTEM

The immune system consists of cellular and molecular components which work together to provide defence against invading microorganisms and destroy antigens.

CELLULAR COMPONENTS OF IMMUNE SYSTEM

The cellular components of immune system consist of several types of cell which arise from pluripotent stem cells in the bone marrow. These immune cells are derived through two main lines of differentiations – the lymphoid lineage producing lymphocytes and the myeloid lineage producing phagocytes (monocytes, macrophages and neutrophils) and other cells (see for detail Chapter 36). The cellular components involved in the adaptive immune response and their lineage relationships are outlined in Figure 56.1 and soluble growth and activation factors (cytokines) released from various cell populations are given in Table 56.2.

I. LYMPHOID LINEAGE

Lymphocytes

Lymphocytes are the central cells of the immune system responsible for acquired immunity, diversity, memory and self-recognition. These are small round cells present mainly in blood, lymph nodes, spleen, thymus and bone marrow. Although most of the lymphocytes in normal lymphoid tissue look alike when studied under the microscope, these cells actually are diverse mixture of cell populations, each with characteristic properties and functions. These cell populations are identified mainly on the basis of characteristic cell surface proteins. Broadly lymphocytes have been divided into two major populations – T lymphocytes and B-lymphocytes. Natural killer cells (or NK cells) constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes.

1. T-Lymphocytes

The T-lymphocytes or T cells are derived originally from pluripotent haematopoietic stem cells and pre-processed or differentiated in the thymus gland (so called T lymphocytes). They can be distinguished from other lymphocyte types, such as B cells and natural killer cells by the presence of a special receptor on their cell surface called T cell receptors (TCR). The T-Lymphocytes are predominantly circulating lymphocytes population accounting for up to 80% of lymphocytes in the peripheral blood. They are primarily responsible for forming activated lymphocytes those provide immunity. The T-lymphocytes are produced at high rate (10^9 per day) and express a large number of protein molecules on their surface. Depending on the type of surface molecules and their role in immunoregulation, T-cells are further divided into several populations of cells which are identified by the systemic nomenclature called CD (cluster designations) system and a number i.e. CD_4 cells, CD_8 cells, CD_{16} cell (CD numbers are actually given to molecules present on the surface of cell and

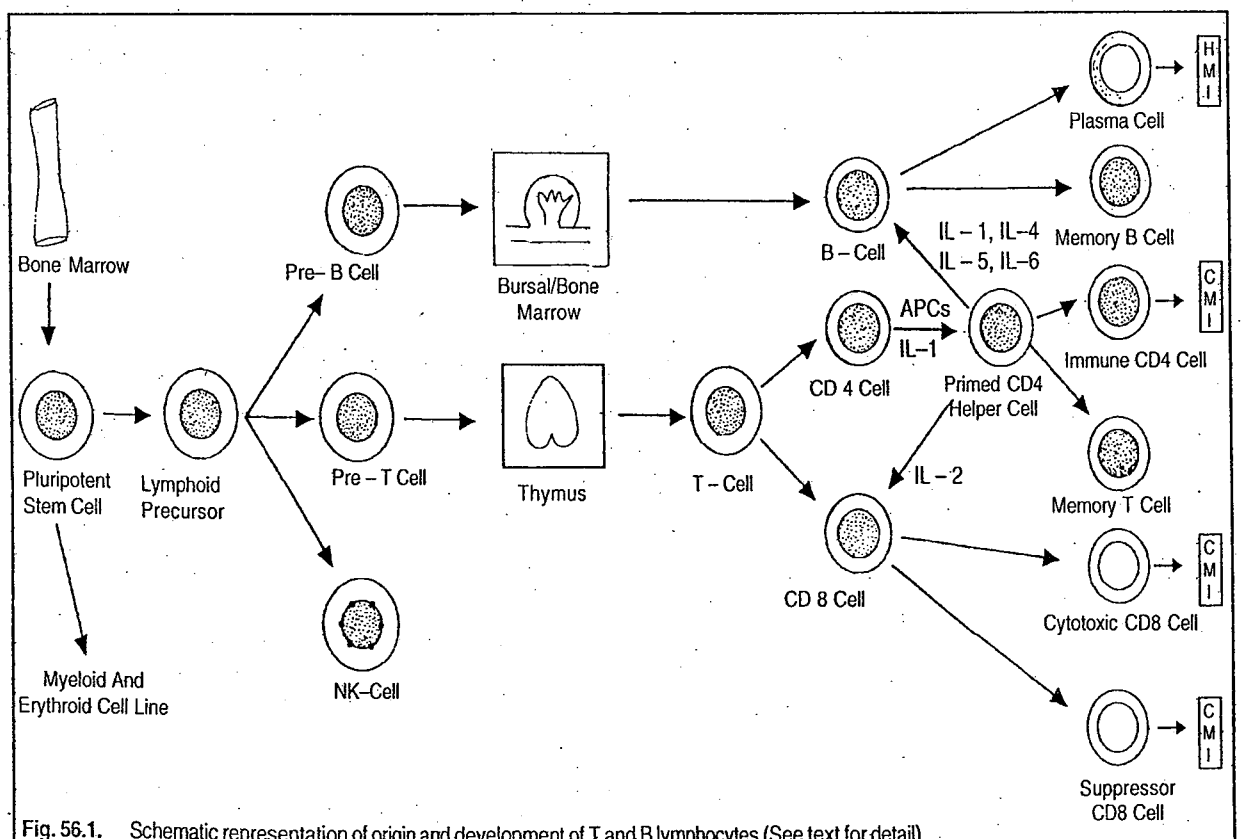


Fig. 56.1. Schematic representation of origin and development of T and B lymphocytes (See text for detail).
Abbreviations : NK cell = natural killer cell ; T Cell = T lymphocyte cell ; B Cell = B lymphocyte cell ; APCs = antigen presenting cell ; IL = interleukin; CMI = cell mediated immunity ; HMI = humoral immunity.

T-cells are recognised by the type of molecule). Some of these CD molecules (e.g. CD_4 and CD_8) are immunologically very important.

Immune Response

The immune response mediated by T-cells is largely due to the presence of highly specific antigenic receptors abbreviated as TCRs (T cell receptors) on their cell surface. The TCR recognises antigen only when the antigen is associated with the major histocompatibility complex (MHC) on surface of antigen presenting cells (APCs) such as dendritic cells, macrophages and other cell types expressing MHC-class I and class II antigens. The binding between TCR and antigen is of relatively low affinity and is degenerate i.e. many TCRs recognize the same antigen and many antigens are recognized by the same TCR.

Normally when an antigen is present in body, it is recognised by the APC as foreign and is subsequently phagocytosed. The APC then processes the antigen and exhibits it on the cell surface in a groove made by major histocompatibility complex molecules on antigen presenting glycoprotein molecules (Figure 56.2). Peptides derived from endogenous proteins (including those synthesised in response to viral stimuli) are expressed with class II MCH molecule on the cell surface, whereas exogenous proteins are expressed bound to a class I MHC molecule. The APC then migrates to T cell area of the lymph node and presents the antigen to unprimed T-cells. If the APC contacts a T-cell with a receptor that recognises the antigen associated with MHC, it becomes activated. Once activated, T cells are triggered to differentiate and divide leading to release of soluble mediators (cytokines) those perform as effectors of the immune system. Progeny cell originating from activated T-cells are CD_4 helper cell, CD_8 cytotoxic cells, CD_8 suppressor cells and memory T cells.

i. ***Helper Cells/CD_4 T cells:*** Helper T cells (CD_4 T cells, Th cells) constitute more than $3/4^{th}$ of the total number of T-cells. These cells are unusual in that they have no cytotoxic or phagocytic activity. However, as their name implies, they help or regulate the functions of immune system mainly by forming a series of protein mediators called cytokines. In absence of the cytokines from the helper T cells, the immune system is almost paralysed. As mentioned above, helper cell receptors recognise and bind to the peptide-MHC class II complex of APC cells. Once activated, they proliferate and become primed as either Th_1 or Th_2 CD_4 cells (also known as Type 1 or Type 2 helper T cells, respectively), which modulate further responses of both the cell mediated and humoral immunities.

The Th_1 subsets of CD_4 produce cytokines like IL-2, interferon γ (IFN-γ) and cytotoxin (TNF-β) within a few hours after stimulation by antigen and are responsible for generation of memory cell and activation of cell-mediated immune responses such as delayed hypersensitivity reaction and macrophage activation. Although Th_1 can also promote B cell proliferation and immunoglobulin secretion, they do not stimulate specific antibody formation. In contrast, the Th_2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 several days after exposure to antigen and stimulate B cell proliferation and immunoglobulin secretion but have no effect on delayed hypersensitivity and other cell-mediated reactions.

ii. ***Cytotoxic T cells:*** Cytotoxic T cells (T_C cells, cytotoxic CD_8 cell) are T cells which are capable of killing microorganisms and, at times, even some of the body's own cells. These cells are activated by interaction with an antigen-class I MHC complex on the surface of altered self-cell (e.g. virus infected cell) in presence of appropriate cytokines. After binding, the cytotoxic T cell secretes hole-forming proteins called performins which make large round holes in the membranes of the target cell. In addition, the cytotoxic cell releases cytotoxic substances directly into the target cell. These cause swelling and lysis of target cells. Some of the cytotoxic T cells are lethal to tissue cells those have been invaded by viruses. Other

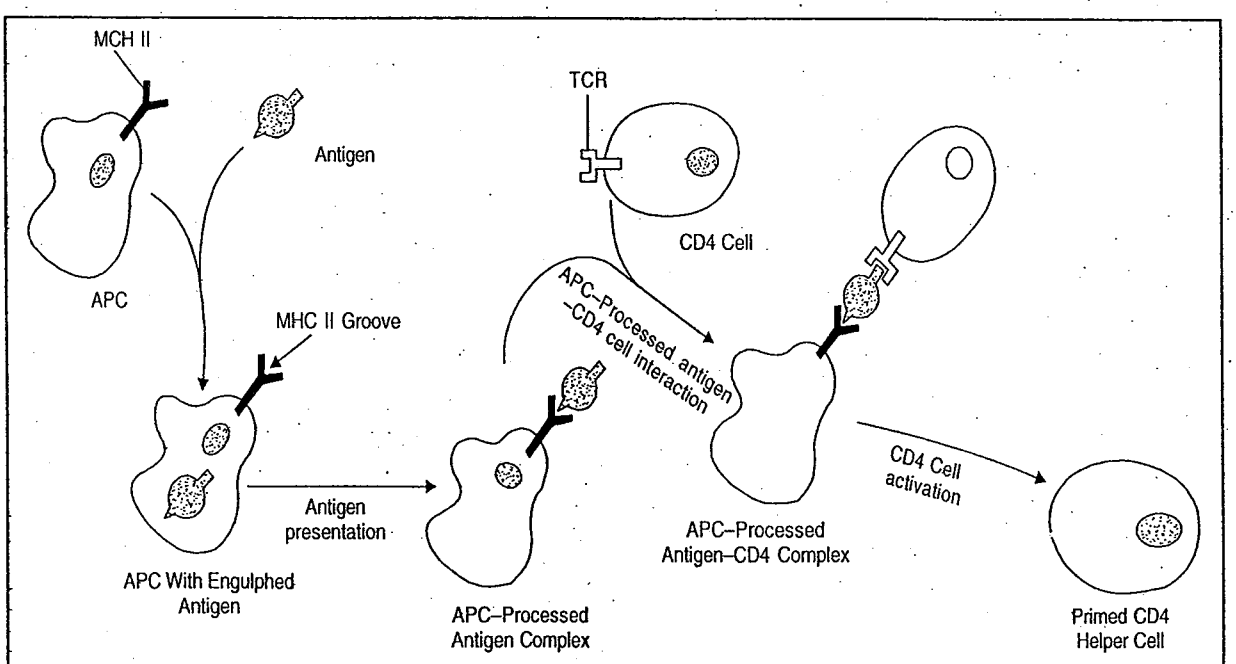


Fig. 56.2. Schematic representation of sequence of events leading to activation of CD 4 helper cell (See text for detail).
Abbreviations : APC = antigen presenting cell ; MHC = major histocompatibility complex ; TCR = T cell receptor.

targets of cytotoxic T cell may include cancer cells and transplant cells.

iii. ***Suppressor T cells/Suppressor CD_8 cells:*** Suppressor T cell, also called regulatory T cells, are capable of suppressing the functions of both cytotoxic and helper T cells. It is believed that they regulate the activities of other cells, keeping them from causing excessive immune reactions which might be severely damaging to the body. It has been suggested that when helper CD_4 cells activate the suppressor T cell, these cells in turn act as a negative feedback controller of the helper T cells.

iv. ***Memory T cells:*** Memory T cells are a subset of antigen-specific T cells which provide a long-term mechanism for a rapid immune response on re-exposure to the antigen. These cells are produced along with cytotoxic T cells when the antigen activates T cells and persist long-term after an infection has resolved. Memory T cells are similar to those of original activated T cells and are preserved in the lymphoid tissue to become additional T lymphocytes of that specific clone. Therefore on subsequent exposure to the same antigen, the release of activated T cells occurs far more rapidly and much more powerfully than in the first response (primary response).

2. B-Lymphocytes

B-Lymphocytes or B-cells are also derived from pluripotent haematopoietic stem cell but are processed in the bone marrow in late foetal life and after birth. This population of cells was first discovered in birds in which the processing occurs in the bursa of Fabricus (so they are called B-lymphocytes). The mature B-lymphocytes are characterised by their surface immunoglobulins (antibodies). These immunoglobulins are made by cells themselves and are inserted into surface membrane, where they act as specific antigenic receptor called B cell-receptor (BCR). Each B cell is covered with about 200,000 to 500,000 BCRs. Appropriate interaction between the antigen and the membrane bound BCR on an unprimed B cell, together with activated T cell (CD_4 helper cell along with its cytokines) and macrophage interactions, induces proliferation and differentiation of B cell into plasma cell and memory cells.

i. ***Plasma cells:*** Plasma cells (Plasma B cells) are ovoid B-lymphocytes, which lack membrane bound antibody activity. They synthesise and secrete one of the five classes of antibodies – IgE, IgA, IgM, IgD or IgE. These antibodies are synthesised at very high rate (about a million molecules per hour for each plasma cell) and secreted rapidly into lymph and then carried to the circulating blood. The immunoglobulins (antibodies) are capable of combining and destroying the antigenic agent. The plasma cells are short lived cells and undergo apoptosis when the inciting agent that induced immune response is eliminated.

ii. ***Memory cells:*** Similar to memory T cells, the memory B cells form a reserve of antigen-sensitive cells which provide a long-term mechanism for rapid immune response on re-exposure to the antigen. On re-exposure to the antigen, they proliferate and differentiate into plasma cell thereby producing a secondary immune response (response produced on re-exposure to antigen), which is much greater than the primary immune response (response produced after first exposure to antigen). The lag period of secondary immune response is shorter and the IgG is produced mainly in preference to the IgM that is characteristic of primary response (Figure 56.3). The initial response (primary response) takes 8-14 days, whereas secondary response occurs within 1-3 days of antigen presentation.

3. Natural Killer (NK) Cells

Natural killer cells (or NK cells) are a type of cytotoxic lymphocytes which constitute a major component of the innate immune system. The NK cells in most mammals are large, granular non-phagocytic lymphocytes. The NK cells and T cells probably arise from a common precursor in the bone marrow, but unlike T cell the NK cells do not undergo pre-processing in the thymus. They constitute about 15% of peripheral blood lymphocytes and are mainly found in the secondary lymphoid organs. Natural killer

Table 56.1: Important features of T and B lymphocytes.

Feature	T-Cells	B-Cells
1. Site of processing	Thymus.	Bone marrow, bursa, Peyer's patches.
2. Distribution	Lymph node paracortex, spleen periarteriolar sheath.	Lymph node cortex, spleenic follicles.
3. Circulation	Yes	No
4. Antigen receptors	TCR-protein heterodimer associated with CD_3, CD_4, or CD_8.	BCR-immunoglobulin.
5. Antigen recognised	Processed antigen proteins on MHC.	Free foreign proteins.
6. Tolerance induction	Easy	Difficult
7. Progeny cells	Effector T cells (immune CD_4 cell, cytotoxic CD_8 cell, suppressor CD_8 cell, memory cell).	Plasma cells and memory B cells.
8. Secreted products	Cytokines.	Immunoglobulins.
9. Antigen processing and presentation	No.	Yes.
10. Type of immunity	Cell mediated.	Humoral.

Abbreviations: T cell = T-lymphocyte cells; B-cell = B-lymphocyte cells; BCR = B-cell receptor; TCR = T-cell receptor.

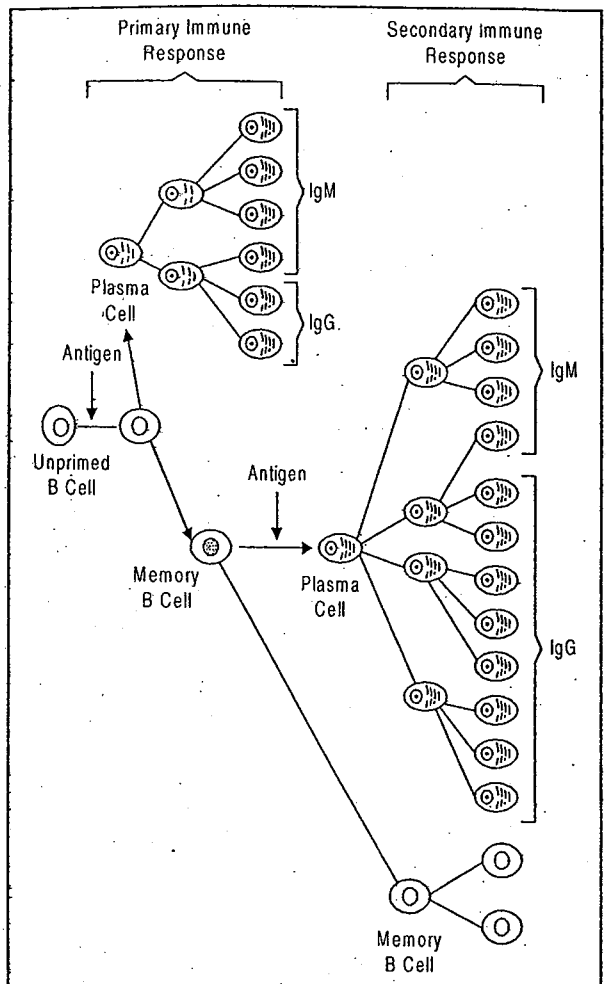


Fig. 56.3. Schematic representation of primary and secondary immune response mediated by B-cells (see text for detail).

cells do not have antigen-binding receptors and therefore lack immunological specificity and memory. However, they are able to kill tumour, virus-infected and some normal cells in the absence of previous sensitisation. Their recognition of target cells occur probably by means other than antigen-receptor activity like they recognise and selectively kill cells which fail to express, or express very reduced amount of MHC class I molecules. Similar to cytotoxic T cells, NK cell mediated killing occurs through release of perforins and cytokines (TNF-α and TNF-β).

II. ERYTHROID AND MYELOID LINEAGE

A. Mononuclear Cells

1. Macrophages

Macrophages are mononuclear phagocytic cells which originate in the bone marrow from pluripotent haematopoietic stem cell (see Chapter 36 and Figure 36.3). They originate first as immature macrophages in blood called monocytes, which form about 5% of the total blood leucocytes. Large portions of these immature cells (monocytes) enter tissues, become mature, acquire ability to fight infectious agents, and are called tissue macrophages or simply macrophages. A majority of macrophages are stationed at important tissues where microbial invasion or accumulation of dust is likely to occur. Each type of macrophage, determined by its location, has a specific name i.e. those lining sinusoids of liver are called Kupffer cells, found in connective tissues are histocytes, and in brain are microglia. Large number of macrophages is also found in alveoli and sinusoids of the spleen, bone marrow and lymph nodes. These mature macrophages remain attached to the tissues for months or even years until they are called to perform specific functions. Macrophages may assume a wide variety of shapes in response to their environment.

Functions of macrophages

Macrophages perform a number of important functions in the inflammation and immune responses. These functions may include phagocytosis, secretion of molecules, processing of antigen and repair of tissue damage (Figure 56.4).

*a. **Phagocytosis:*** Macrophages can phagocytose large quantities of bacteria, viruses, necrotic tissue or foreign particles in the tissues. Phagocytosis by macrophages is similar to the process of neutrophils. When appropriately stimulated, they can break away from the attachments in tissue and once again become mobile macrophages, which respond to chemotaxis and other stimuli from inflammatory processes. Macrophages then destroy ingested organisms/particles through various mechanisms such as secretion of lysosomal enzymes and oxygen metabolites.

*b. **Secretion of molecules:*** Macrophages can synthesise and secrete many different proteins. These may include enzymes (e.g. lysosomes, proteases and collagenases), complement components (e.g. C_2, C_3, C_4 and C_5), cytokines (e.g. IL-1, IL-6, IL-12 and TNF-α), clotting factors and prostanoids. Role of cytokines, in particular, is important in regulation of immune responses. Activated macrophages also produce nitric oxide (NO), which can react with superoxide anion to produce toxic derivatives (e.g. NO_2, NO_2^-, N_2O_3, $ONOO^-$ and NO_3) which help to kill intracellular parasites and ingested bacteria.

*c. **Processing of antigen:*** As discussed earlier, macrophages can act as antigen-presenting cell (APC) in cell-mediated immune response. Macrophages can process the phagocytised antigen and present the peptide fragments attached to the specialised MHC molecule. Macrophages are the most important and most accessible antigen-processing cells.

*d. **Tissue repair:*** Macrophages are essential for the tissue repair and healing. This is performed by phagocytose killing and removing the debris of invading organism or damaged tissue. When the damaged tissue is removed, macrophages act as a source of growth factors for fibroblasts and stimulate them to secrete collagen. They also regulate synthesis of connective tissue matrix proteins and secrete molecules which regulate growth of new blood vessels.

B. Polymorphonuclear Cells/Granulocytes

1. Neutrophils

Neutrophils (Polymorphonuclear neutrophils) are the important cell type of the myeloid system constituting about 60-70% of the blood leucocytes in most carnivores, but only about 20-30% in ruminants. These are formed in the bone marrow and then migrate to the blood stream and later move into the tissues. They contain granular cytoplasm with an irregular segmented nucleus. Neutrophils possess two types of granules, the primary granules (azurophilic) and the secondary granules (specific granules).

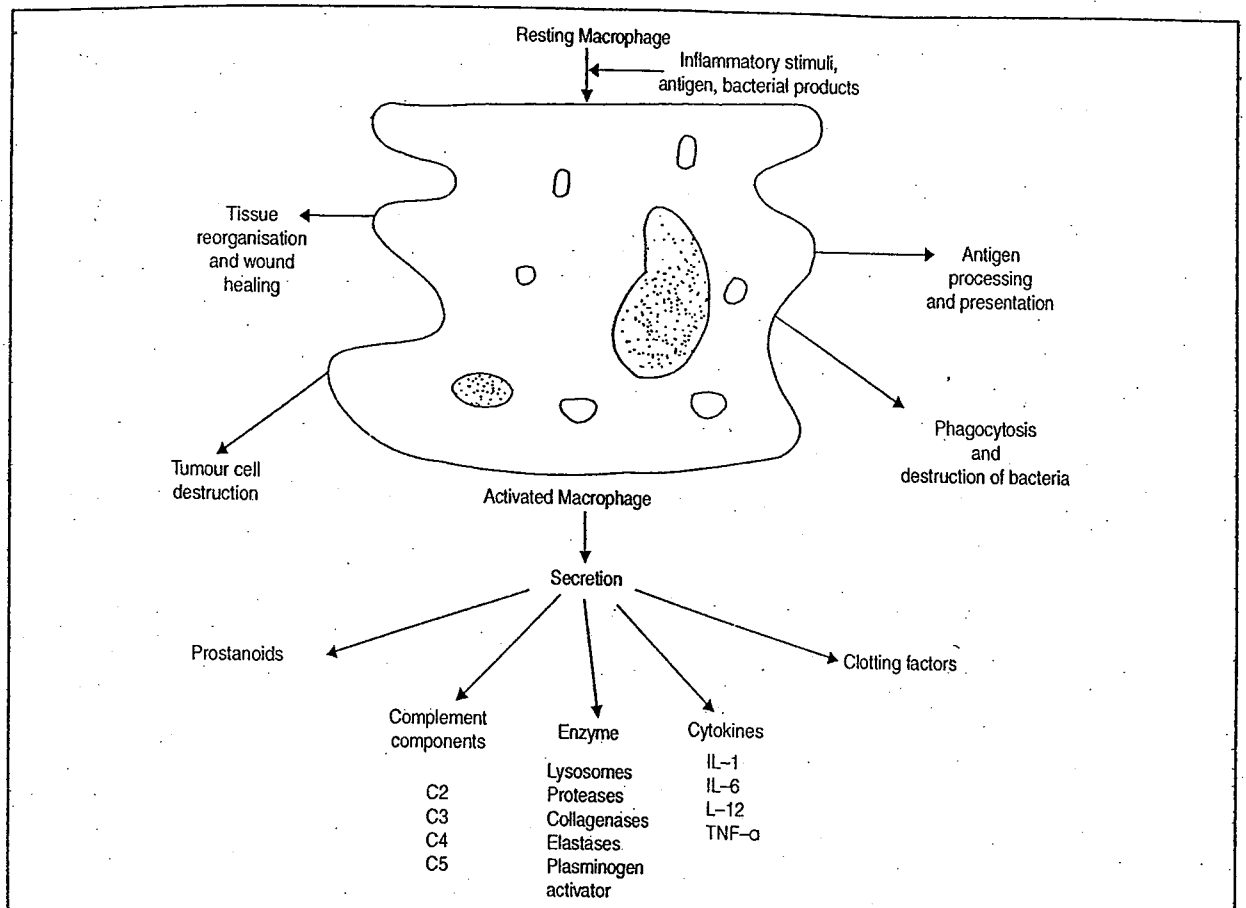


Fig. 56.4. Important functions of macrophage (See text for detail).
Abbreviations : IL = interleukin ; C = complement component ; TNF-α = tumour necrosis factor-alpha.

Primary granules contain bactericidal enzymes such as lysozyme and myeloperoxidase, neutral proteases such as elastase, and acid hydrolase such as beta-glucuronidase and cathepsin B. Secondary granules contain lysozyme and collagenase, which destroy bacterial cell wall and degrade connective tissue, respectively. Mature neutrophils cannot synthesize large amounts of proteins because they have very few ribosomes or rough endoplasmic reticulum.

The most important function of the neutrophils is phagocytosis, which occurs in four stages – chemotaxis, adherence, ingestion and digestion.

i. ***Chemotaxis:*** It is the attraction and movement of cell towards the source of certain chemicals. During this process, neutrophils leave the blood by diapedisis and migrate into the tissues in response to production of many different chemotactic agents by bacterial invasion and tissue injury. These chemotactic agents may include peptides like C5*a* (generated by complement system) and fibrinopeptide B (derived from fibrinogen), and molecules like platelet factor 4 (released by platelets) and leukotriene B (synthesised from phospholipids). Increased adhesiveness of endothelial cells forming the blood vessel walls helps in movement of neutrophils through expression of adhesive proteins called selectins and integrins.

ii. ***Adherence:*** Neutrophils those enter the tissues possess the capability to bind to foreign particles. This happens mainly by coating of the foreign particles with a type of proteins called opsonins. The process of opsonisation is essential because both neutrophils and particles suspended in body fluids usually have a negative charge and so repel each other.

iii. ***Ingestion:*** As neutrophils approach a chemotactic source and attach to the particle to be phagocytised, it projects pseudophilia in all directions around the particle and forms an enclosed chamber. Then the chamber invaginates to the inside of cytoplasmic cavity and breaks away from outer cell membrane to form a free-flowing phagocytic vesicle called phagosome. A neutrophil can usually phagocytise 5 to 20 bacteria before the neutrophil itself becomes inactivated and dies. The ease of ingestion depends on the type of particle.

iv. ***Destruction:*** Once the foreign particle has been ingested, it is usually digested by intracellular enzymes such as lysozymes. In addition, destruction of ingested particles may occur through several powerful oxidising agents like superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ions (OH^-). Defensins, the small proteins in primary granules, attack plasma membrane of many different bacteria, fungi and enveloped viruses and destroy them.

Unlike macrophages, neutrophils survive for only a few days and can undertake only a limited number of phagocytic events. Since neutrophils usually destroy all ingested foreign particles, they cannot process antigen for presentation to antigen-sensitive cells.

2. Eosinophils

Eosinophils constitute about 2% of all the blood leucocytes. Eosinophils originate from pluripotent stem cell in the bone marrow and spend a short period (half-life ~0.5 hour) circulating in the blood stream. They subsequently migrate into tissues, where they a have life of about 12 days. Eosinophils are weak phagocytes whose primary function is to destroy foreign material. The granules of eosinophils contain no lysozyme, but phosphatase and peroxidase.

Phagocytosis by eosinophils, like neutrophils, can be divided into chemotaxis, adherence, ingestion and digestion. Histamine, some metabolites of arachidonic acid and certain peptides attract eosinophils to target sites. Although they can phagocytose small particle similar to that of neutrophils, the eosinophils are more useful for extracellular destruction of large parasites. Therefore, eosinophils are produced in large quantities in animals with parasitic infections. Eosinophils also have a special potential to collect in tissue where allergic reactions have occurred such as in the peribronchial tissue of the lungs in individuals with asthma and in the skin after allergic skin reactions. Eosinophils are also capable of producing reactive oxygen species like superoxide and peroxide, lipid mediators like eicosanoids from the leukotriene (e.g. LTC_4, LTD_4, LTE_4) and prostaglandin (e.g. PGE_2), and cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13 and TNF alpha.

3. Basophils

Basophils are derived from the pluripotent stem cells in bone marrow and constitute about 0.5% of blood leucocytes. They are not normally found in extracellular tissues. They may, however, infiltrate tissues under the influence of lymphocytes and may participate in many specific kinds of inflammatory reactions, particularly those which cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. When activated, basophils degranulate to release histamine, proteoglycans (e.g. heparin and chondroitin), and proteolytic enzymes (e.g. elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes, and several cytokines.

C. Other Cells

1. Platelets

Platelets or thrombocytes are minute round or oval discs formed in the bone marrow from megakaryocytes, the large cells of the haematopoietic series. Although platelets do not have nuclei and cannot reproduce, they have functional characteristics of whole cells. In addition to blood clotting mechanisms (see Chapter 35), platelets play role in immune responses. They express class I MHC and receptors of IgG. They are also suggested to modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines and other inflammatory mediators.

2. Mast Cells

Mast cells (also known as mastocyts) are large, round cells, which are distributed throughout the body in connective tissues (also mucosal cells). They contain a large number of granules in their cytoplasm, which contain heparin, histamine (in some species) and serotonin and can produce different prostaglandins and leukotrienes as well as platelet activating factor. Mast cells are major sources of cytokines including IL-4, IL-5, IL-6, IL-13 and TNF-α. The mast cells play important role in some types of allergic reactions because the type of antibody that causes allergic reactions (IgE) has a special propensity to become attached to mast cells (See also Chapter 46).

3. Dendritic Cells

Dendritic cells are a specialised population of mononuclear cells involved mainly in processing and presentation of antigen to the T-cells. They are located throughout the body but especially in lymphoid organs. They possess long, filamentous cytoplasmic processes called dendrites. They are more potent antigen presenting cells than macrophages and B cells, which need to be activated before they act as APCs. Several different types of dendritic cells are found in the body including Langerhans cell in epidermis and mucous membranes, interstitial dendritic cell in heart, liver, lungs, kidneys and GI tract, follicular dendritic cells in lymphoid tissues, and circulating dendritic cells in blood and lymph.

MOLECULAR COMPONENTS OF IMMUNE SYSTEM

I. CYTOKINES

Cytokine is a generic name given to a variety of regulatory and growth proteins secreted by cells of the inflammatory and immune system. These proteins modulate the immune response mainly by signalling between cells. Cytokines were originally named after their cell of origin or the bioassay used to identify them like cytokines secreted by lymphocytes were named lymphokines and those produced by monocytes/macrophages as monokines. Presently, the cytokines are categorised into families based on homology in primary amino acid sequence, three dimensional structure, induction mechanisms, chromosomal locations, similarities in receptor type, and functional homology. These families have been named interleukins, interferons, tumour necrosis factors, haematopoietic growth factors and chemokines.

The cellular sources of most cytokines are diverse with production occurring in many types of cell. Most cytokines are not constitutively produced but are secreted after activation of cells by viral, bacterial and parasitic infections. Normally, they have autocrine and paracrine (rather than endocrine) effects, with little or no detectable circulating level. Because of the high cytokine-receptor affinity, cytokines are effective in the picogram or nanogram per ml range.

Mechanism of action

Structurally, cytokines can be classified as proteins, peptides or glycoproteins. Each cytokine has a matching cell-surface receptor. The binding of the cytokine to its receptor initiates the intracellular signal transudation cascade, which then alter cell functions by modulation of several genes and their transcription factors. This results in the production of other cytokines and an increase in the number of surface receptors for other molecules. Cytokines also form a complex feedback network either by altering the expression of other cytokines or the suppression of their own effect by feedback inhibition. In addition to cell surface receptors, soluble receptors may also be involved in cytokine transport or inhibition of cytokine activity.

Interleukins (ILs)

Interleukins are a very heterogeneous mixture of proteins/signalling molecules which regulate interactions between lymphocytes and other leucocytes. The term interleukin derives

Table 56.2: Selected cytokines and their role in immune response.

Cytokine	MW (kDa)	Main source(s)	Major targets	Response
Interleukins				
IL-1	17	Macrophages, B cells, NK cells, macrophages, monocytes, vascular endothelium.	T cells, B cells, NK cells, macrophages, neutrophils, stem cells.	Stimulation of early bone marrow stem cells and lymphocyte precursors; stimulation of Th_2 cells; NK cell activation; induction of IL-1, IL-6, IL-8, TNF, GM-CSF, and prostaglandin E_2 production by macrophages; inflammation.
IL-2	15	Th_1 cells.	T cells, B cells, NK cells, macrophages.	T cell proliferation and generation of cytotoxic T cells; activation of B cell, NK cells and macrophages.
IL-3	25	T cells (both Th_1 and Th_2), eosinophils, mast cells, endothelium.	Haematopoietic cells, stem cells, mast cells, macrophages.	Growth and maturation of bone marrow lineage cells; growth of mast cells; stimulation of macrophage phagocytosis and cytotoxicity.
IL-4	20	Th_2 cells, Mast cells, NK cells, macrophages.	T cells, B cells, mast cells, macrophages.	Growth and differentiation of B cells; activation of T cells and macrophages.
IL-5	18	Th_2 cells, mast cells, eosinophils.	B cells, T cells, eosinophils.	Growth and differentiation of eosinophil precursors in bone marrow; stimulation of B cell growth and induction of cytotoxic T cell activity.
IL-6	26	Macrophages, T cells, B cells, fibroblasts, astrocytes, dendritic cells.	T cells, B cells, hepatocytes, bone marrow, stroma cells.	Stimulation of IL-2 and IL-2R production and T cell differentiation; proliferation of bone marrow and plasma cells; induction of acute phase reaction, haematopoiesis, inflammation.
IL-7	25	Bone marrow, spleen cells, thymic stromal cells.	T cells, B cells, thrombocytes, mono-cytes, lymphoid stem cells.	Proliferation of pre-B cells and T cells; regulation of lymphoid stem cells.
IL-8	8	Macrophages, fibroblasts, lymphocytes, epithelial cells, hepatocytes.	T cells, neutrophils, basophils.	Chemotactic for B and T cells and neutrophils; activation of neutrophils.
IL-9	39	Th_2 cells.	T and B cells	Induction of growth of helper T cells and bone marrow derived mast cells; synergistic with IL-4 on B cells.
IL-10	19	Th_2 cells, B cells, mast cells and macrophages.	Th_1 cells, B cells, macrophages, NK cells, mast cells.	Inhibition of Th_1 and NK cells function; suppression of activated macrophages.
IL-11	24	Bone marrow stromal cells and fibroblasts.	B cells	Stimulation of B cells in association with IL-6; synergistic with IL-3; production of acute phase protein production; formation of osteoclast.
IL-12	75	Macrophages, monoocytes, B cells.	Th_1 cells, NK cells.	Stimulation of Th_1 activity by induction of IL-2 and IFN-γ secretion; proliferation of T cell and NK cell activity and cytotoxicity.
IL-13	10	Th_2 cells, mast cells.	B cells, TH2-cells, macrophages.	Inhibition of macrophage functions and suppression of IL-1, IL-6, IL-12 and NO production; stimulation of B cell growth and secretion of IgG and IgE.
IL-14	53	T cells, B cells	B cells	Induction of B cell proliferation; inhibition of immunoglobulins secretions.
IL-15	15	Macrophages, epithelial and fibroblast cell lines.	T cells, NK cells.	Proliferation of cytotoxic T cells and helper T cells; growth of T cells and NK cells.
IL-16	14	Lymphocytes, epithelial cells, eosinophils, CD8+ T cells.	Th-cells	Chemoattractant for CD4 T cells, monocytes, and eosinophils.

IL-17	35	T cells	Epithelium, endothelium	Pro-inflammatory; angiogenesis, increase production of inflammatory cytokines.
Interferons				
IFN-α	16–25	Macrophages, lymphocytes, monocytes.	Many cells	Activation of macrophages, T cells and NK cells; enhancement of class I MHC expression; inhibition of viral growth in virus infected cells.
IFN-β	16 – 25	Fibroblasts	Many cells	-do-
IIFN-γ	20 – 25	Th_1 cells, NK cells.	B cell, T cell, NK cell, macrophages	Stimulation of B cell production; activation of macrophages and T cell; expression of MHC; inhibition of viral growth in virus infected cells.
IFN-ω	16 – 25	Trophoblasts, lymphocytes, monocytes.	T cells	Activation of macrophages; inhibition of viral growth in virus infected cells.
IFN-τ	16 – 25	Trophoblasts.	T cells	-do-
Tumour Necrosis Factor				
TNF-α (Cachectin)	17	Macrophages, T cells, B cells, fibroblasts, mast cells, NK cells.	Tumour cells, many other cells.	Cytotoxic effect on tumour cells; activation of macrophages; stimulation of inflammatory and immune responses; anti-viral action; growth of many cell lines; causing of cachexia.
TNF-β (Lymphotoxin)	25	T cells.	Tumour cells, many other cells.	Cytotoxic effect on tumour cells; activation of neutrophils, macrophages, B cells and endothelial cells; anti-viral action.
*Growth Factors**				
GM-CSF	18/22	T cells, macrophages, fibroblasts, endothelial cells.	Haematopoietic cells	Bone marrow proliferation and activation of macrophages; stimulation of phagocytosis; expression of MHC class II molecules.
G-CSF	23	Macrophages, endothelial cells, fibroblasts.	Haematopoietic cells	Regulation of granulocyte progenitors into mature granulocytes; stimulation of phagocytic and cytotoxic activity of neutrophils.
M-CSF	40/80	Lymphocytes, macrophages, endothelial cells, fibroblasts.	Monocytes, stem cells.	Proliferation and differentiation of monocytes and macrophages; stimulation of macrophage cytotoxicity.
Chymokines				
MIP-1		Macrophages, T cells, B cells, mast cells, neutrophils.	Monocytes, eosinophils, B cells, T cells.	Chemotactic for T cell, B cell, eosinophils.
MCP-1		Macrophages, T cells, fibroblasts, endothelial cells.	Monocytes.	Chemotactic for monocytes; stimulation of release of lysosomal enzymes.
RANTES protein		T cells, macrophages.	Monocytes, eosinophils, T cells, basophils.	Chemotactic for monocytes, eosinophils and T cells; activation of T cells; stimulation of histamine release from basophils.

* = Several interleukins (e.g., ILs- 3, 7, 11, 14, 15) also function as growth factors.

Abbreviations: IL = interleukin; T cells = T lymphocyte cells; B cells = B-lymphocyte cells; NK cells = natural killer cells; Th cells = helper T cell; NO = nitric oxide; Ig = immunoglobulin; IFN = interferon; TNF = tumour necrosis factor; GM-CSF = granulocyte-macrophage colony stimulating factor; G-CSF = granulocyte colony stimulating factor; M-CSF = macrophage colony stimulating factor; MIP-1 = macrophage inflammatory protein 1; MCP = monocyte chemoattractant protein; MHC = major histocompatibility complex; R = receptor; α = alpha; β = beta; γ = gamma; ω = omega; τ = tau.

from words *inter-* meaning a method of communication and *– leukin* meaning those derived from leucocytes and/or act on leucocytes. There are currently 17 common families of interleukins, which are numbered sequentially in the order of their discovery and have very little in common except their name. Interleukins are mainly produced by T and B lymphocytes, macrophages, fibroblasts and other stromal cells. Secretion by these cells is inducible in response to a variety of natural and artificial stimuli. With appropriate stimulation, interleukins are secreted in a cascading network regulating activation, proliferation, differentiation or secretion of antibody or other interleukins by a variety of cells. The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages and endothelial cells.

The effects of interleukins are mediated by binding to specific receptors expressed on the cell membranes of responding target

cells. Interleukins may act on a variety of cellular targets and may have an autocrine effect (effect on the same cell that produce them), a paracrine effect (effect on cells in very close proximity to the cell of origin), or an endocrine effect (effect on cells in distant locations). These effects may include stimulation or inhibition of cell division or differentiation, stimulation of production or expression of new receptors, and stimulation or inhibition of new protein synthesis. Most cytokines act on a large number of different target cells, perhaps inducing different responses in each one. Important functions of interleukins include enhancement or suppression of various cells of immune system (e.g. IL-1, IL-2, IL-4, IL-9, IL-10, IL-12, IL-13 and IL-17), haematopoietic growth factor activity (e.g. IL-3, IL-11 and IL-13), and regulation of leucocyte functions (e.g. IL-5 and IL-8). Some are growth factors for cells of the immune system (e.g. IL-7, IL-11, IL-14 and IL-15), whereas others enhance the acute phase response (e.g. IL-1 and IL-6). A brief summary of interleukins and their immune responses is given in Table 56.2.

Interferons (IFNs)

Interferons (IFNs) are glycoproteins synthesised in response to the presence of pathogens such as viruses, bacteria or parasites, or tumour cells. Five types of interferons are recognised – interferon alpha (IFN-α), interferon-beta (IFN-β), interferon gamma (IFN-γ), interferon-omega (IFN-ω), and interferon tau (IFN-τ). The IFN-α, IFN-β and IFN-ω are generally called the 'Type I interferon', whereas IFN-γ is called 'Type II interferon'. All type I IFNs bind to a specific cell surface receptor complex known as the IFN-α receptor (IFNAR).

Interferons alpha and gamma are important in the activation of macrophages and T-lymphocytes (Table 56.2). Interferon gamma also increases MHC class I expression on tumour cell lines and induces appearances of MHC class II on endothelial cells, myeloid cells, some dendritic cells and macrophages. Interferons inhibit the growth of almost all known viruses by interfering with viral RNA and protein synthesis. They also have several effects on neoplastic cell including modulation of oncogene expression. Production of interferons predominantly occurs in response to microbes, such as viruses and bacteria, and their products.

Tumour necrosis factors (TNFs)

Tumour necrosis factors are two closely related cytokines – the tumour necrosis factor alpha (TNF-α) and tumour necrosis factor beta (TNF-β). As their name implies, they both have the ability to cause cytotoxic effect on some tumour cells. The TNF-α is secreted by macrophages, T-cell, B-cells and fibroblasts and can act on almost all nucleated cells. It acts as mediator of many immune responses, regulates the growth of many cell types and activates macrophages. The TNF-β, produced by Th_1 cells, mainly activates neutrophils, macrophages, endothelial cells and B cells.

The primary role of TNF is in the regulation of immune cells. TNF is able to induce apoptotic cell death, inflammation, tumorigenesis and viral replication. Dysregulation of TNF production has been implicated in a variety of human diseases, including major depression, Alzheimer's disease and cancer.

Haematopoietic growth factors

Haematopoietic growth factors are a group of glycoprotein cytokines, which control proliferation and maturation of major blood cells and activate many immune and inflammatory cells. Multiple cell types produce each of these glycoproteins and in many cases affect different cellular targets. These include several interleukins and colony stimulating factors. For example, granulocyte macrophage colony stimulating factor, derived from T cells, macrophages, fibroblasts and endothelial cells, act as growth factor for myeloid progenitor cells (see also Table 56.2). It also induces phagocytosis, activates macrophages and enhances superoxide production and expression of MHC class II molecules. Granulocyte colony stimulating factor (G-CSF) produced by macrophages, endothelial cells and fibroblasts regulates the maturation of granulocyte progenitors into mature neutrophils. Macrophage colony stimulating factor (M-CSF) derived from lymphocytes, macrophages, fibroblasts and endothelial cells induce proliferation and differentiation of monocytes and macrophages and promote macrophage cytotoxicity.

Chemokines

Chemokines are a family of small proteins which play important role in inflammation. They have been subdivided into the alpha and beta subfamilies. The α-chemokines, also called CXC chemokines, include IL-8 that is chemotactic for neutrophils, basophiles and some T-cells. The β-chemokines, also called CC chemokines, include macrophages inflammatory protein (MIP-1), monocyte chemoattractant protein (MCP), and RANTES protein. The MIP-1 attracts helper T cells, cytotoxic cells, B cell and eosinophils, whereas MCP is chemotactic and activating for monocytes and stimulates lysosomal enzymes. The RANTES protein, produced by T cells and macrophages, is mainly chemotactic for monocytes, eosinophils and some T cells. It also activates eosinophils and stimulates histamine release from basophils. Chemokines mediate their biological functions through chemokine receptors, which are G protein-coupled receptors found on the surface of leukocytes.

II. COMPLEMENT SYSTEM

Complement system is a collective term used to describe a number of small proteins found in the blood which help or "complement" the ability of antibodies and phagocytic cells to clear pathogens from an organism. These proteins are generally synthesized by the liver and normally circulate as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade that ultimately results in the destruction of cells or invading organisms. The complement system is part of the innate immune system that is not adaptable and does not change over the course of an individual's lifetime, but can be recruited and brought into action by the adaptive immune system.

The complement system includes over 25 proteins and protein fragments including serum proteins, serosal proteins and cell membrane receptor proteins. Many of these proteins are enzyme precursors and some are control proteins. These complement proteins have been labelled either numerically with the prefix C (e.g. C1, C2, C3, etc) or are designated by the letters of alphabet B, D, P, etc. Out of various complement proteins, 11 proteins designated C1 to C9, B and D are involved mainly in cascade reactions as shown in Figure 56.5. Similar to some other enzymatic chain reactions (e.g. blood clotting reaction), the complement system include a cascade reaction and usually requires a trigger to initiate the reaction chain. The cascade results in highly amplified events which interact with other physiological cascades including

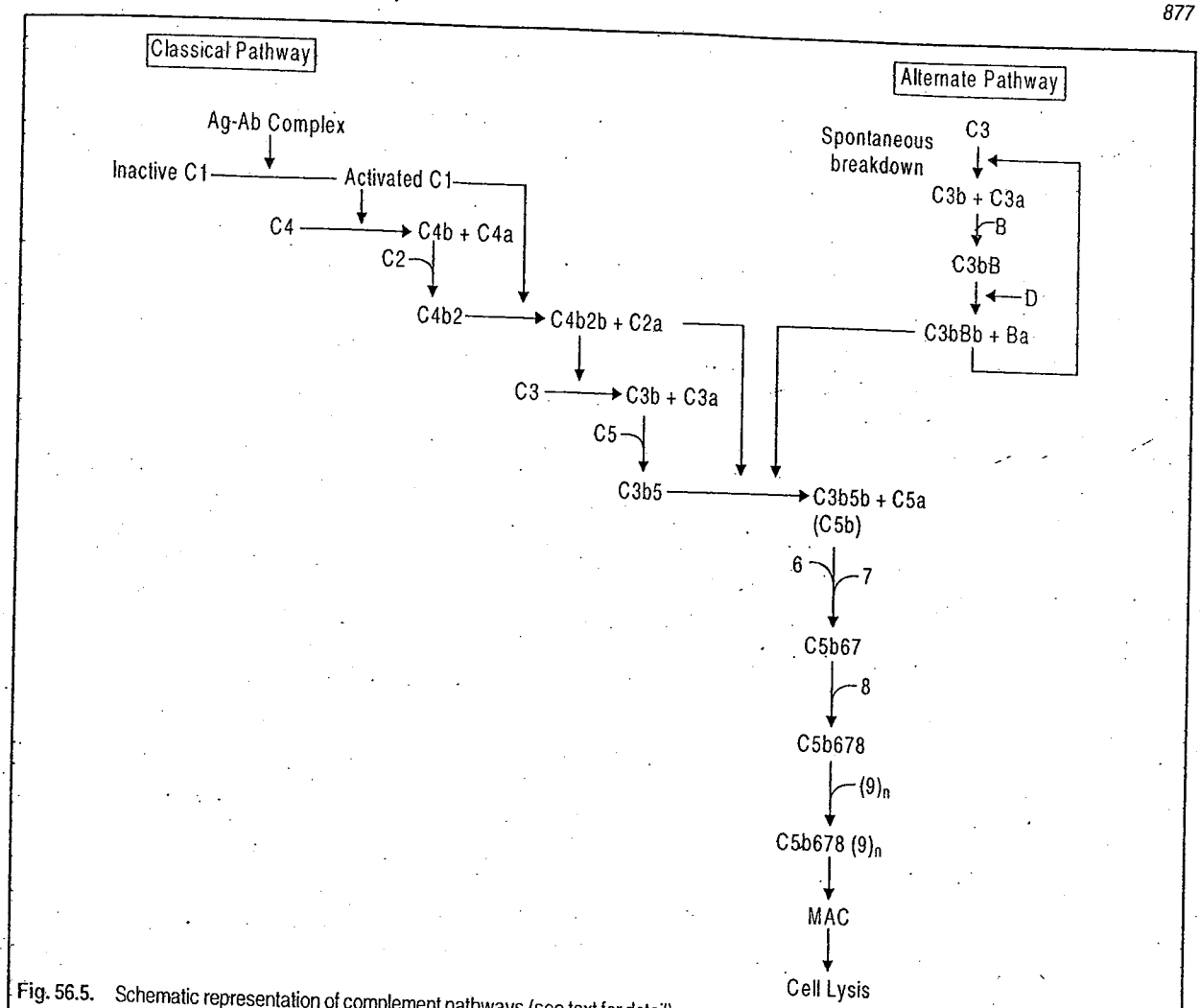


Fig. 56.5. Schematic representation of complement pathways (see text for detail).
Abbreviations : Ag = antigen ; Ab = antibody ; C_1-C_9, B, D = complement components ; MAC = membrane attack complex.

the coagulation pathway, kinin formation and fibrinolysis. Complement is an essential part of body's defences, but it must be carefully regulated because uncontrolled activation may lead to massive cell or tissue destruction.

The complement system may be activated through two main pathways – classical pathway and alternate pathway.

1. Classical pathway

The classical pathway (Classical complement pathway) is initiated by an antigen-antibody reaction. The antigen-antibody complex binds directly with the proenzyme C1 and sets into motion a cascade of sequential reactions (Figure 56.5). The activated C1 acts on C4 and breaks it into a small fragment C4a and large fragment C4b. Complement component C2, a glycoprotein, then binds to C4b to form a complex C4b2. Activated C1 then splits the bound C2 into C2a and C2b, generating a small peptide (C2a) and a complex called C4b2b. The C4b2b complex is a potent protease, which subsequently acts on C3, an important component of complement system, and forms a small molecule C3a and an active molecule C3b. The activation of C3b by C4b2b is a major step, since each C4b2b complex can activate as many as 200 C3 molecules. The C3b molecule then binds to target cell surface.

The next phase of complement system in initiated when C5 binds to surface bound C3b. The resultant complex C3b5 is now susceptible to action of either C4b2b or C3bBb (formed through alternate pathway), which splits off a small peptide called C5a. The part of complex remaining attached to C3b is called C5b. The C5b then binds to C6 and C7 to form C5b67. The C5b67 complex detaches from the surface C3b and enters into the lipid bilayer of nearby cell or microbial membranes. This initiates incorporation of one C8 and multiple (12-19) C9 molecules into the complex to form $C5b678(9)_n$. Binding of several C9 molecules with the complex forms a tubular transmembrane pore called membrane attack complex (MAC). The MAC inserts into a cell membrane and forms a transmembrane channel. Formation of sufficient MACs on the cell membranes causes osmotic lysis of the target cell.

2. Alternate pathway

Sometimes, the complement system is activated without involvement of an antigen-antibody reaction by mechanisms called the alternate pathway (also called alternative or alternative complement pathway). This may occur in response of large polysaccharide molecules in the cell membrane of some invading microorganisms. These substances react with complement component B and D forming activation products which activate component C3. In this pathway, the component C3 breaks down slowly but spontaneously in plasma to form C3a and C3b. The C3b binds to cell surfaces similar to the classical pathway. In the presence of an activating surface, the protein B binds to the C3b to form a complex called C3bB (Figure 56.5). The bound factor B

is then cleaved by another protease called factor D into a soluble fragment called Ba and bound fragment C3bBb. The C3bBb can split C3 and generate C3b, or binds to C3b5b to form final products of the system as mentioned in the classical pathway.

Effects of Complement System

Complement system plays a major role in inflammatory and immune responses. Among various functions, following are important:

a. ***Bacterial cell lysis:*** This is the most important effect of the complement system. As discussed above, bacterial cell lysis is caused by the complex $C5b678(9)_n$, which forms membrane attack complex (MAC). This has a direct effect on rupturing the cell membrane of bacterial and other invading organism. The complement enzymes and other complement products can also attack structures of some viruses and thereby render them nonvirulent.

b. ***Opsonisation and phagocytosis:*** One of the products of complement cascade, the C3b, strongly activates phagocytosis by both neutrophils and macrophages by coating the foreign cells.

c. ***Chemotaxis:*** Activation of complement system generates chemotactic peptides including C5a and C5b67. The C5b67 is chemotactic for neutrophils and eosinophils, whereas C5a attracts neutrophils, eosinophils, basophils and macrophages.

d. ***Activation of mast cell and basophils:*** Complement fragments C3a, C4a and C5a activate mast cells and basophiles causing them to release histamine, heparin and several other mediators. These mediators play important role in inflammatory and immune responses.

e. ***Inflammatory effect:*** In addition to inflammatory effects caused by activation of mast cells and basophiles, some complement peptides contribute directly to the inflammatory responses. The C3a and C5a are called anaphylatoxins because they cause contractions of bronchial and intestinal smooth muscles.

f. ***Immune response:*** In addition to immune effects mentioned above, C3 and its receptors play a role in regulating the immune system. Deficiencies in the classical pathway components are associated with reduced primary antibody responses. The C3 fragments regulate B cell proliferation and formation of antibody.

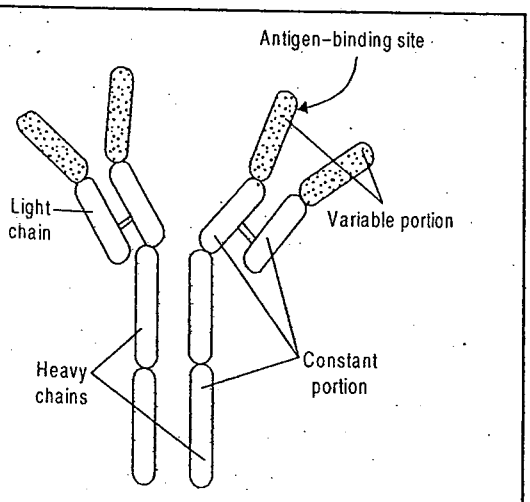


Fig. 56.6. Structure of typical IgG antibody.

III. ANTIBODIES / IMMUNOGLOBULINS

Antibodies, also known as immunoglobulins, are large Y-shaped glycoproteins used by the immune system to identify and neutralize foreign objects like bacteria and viruses. Antibodies are produced by plasma cell and can occur in two physical forms, a soluble form that is secreted from the cell, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B cell receptor (BCR). Antibodies usually constitute about 20 percent of all the plasma proteins and have molecular weights in range of 160,000 - 970,000.

Antibodies are typically made of basic structural units - a Y-shaped structure that is generally composed of combinations of 2 light and 2 heavy chains, though some have combinations of as many as 10 light and 10 heavy chains which give rise to the high molecular weight immunoglobulins. The light and heavy chains are attached by covalent bonds in such a way that each heavy chain is paralleled by a light chain at one of its end (Figure 56.6). The heavy and light chains are divided into a variable (V) region and a constant (C) region. The variable portion or Fab region is located at the amino-terminal ends of the Y arms; it is called variable because the amino acids it contains are different in different antibodies. The amino acids present determine the specificity of the Ig and accordingly the V region attaches specifically to a particular type of antigen. The small site on an antigen to which a complementary antibody may specifically bind is called 'epitope'. The remaining part of antibody (other than variable portion) forms the constant portion or Fc region. The C region contains a relatively constant sequence of amino acids that is distinctive for each Ig isotype. The constant portion determines other properties of the antibody such as diffusivity of antibody in tissues, adherence of antibody to specific structures within tissues, and attachment to complement complex. The antibodies are produced in response to foreign molecules in the body and are capable of specific combinations with the antigens which induced their production.

Types of Antibody

There are several different types of antibodies, which are grouped into different isotypes based on which heavy chain they possess. Five different antibody isotypes or classes are known to exist in mammals, which perform different roles and help direct the appropriate immune response for each different type of foreign object they encounter. These five general classes or isotypes of antibodies are named immunoglobulins G (IgG), M (IgM), E (IgE), A (IgA) and D (IgD).

Immunoglobulin G (IgG)

Immunoglobulin G makes up the majority of total immunoglobulins in body constituting about 75% of the antibodies of normal animal. It has molecular weight of about 180 kDa containing two identical light chains and two heavy chains. It is synthesised and secreted by plasma cells found in the spleen, lymph nodes and bone marrow.

There are 4 subclasses of IgG: IgG1, IgG2, IgG3 and IgG4. They are numbered in descending order of their serum concentration. IgG subclasses differ functionally mainly in their ability to activate complement; IgG1 and IgG3 are most efficient, IgG2 is less efficient and IgG4 is inefficient.

Immunoglobulin G plays a major role in the antibody-mediated defence mechanisms mainly because of its high concentrations in the blood. Further its small size and high solubility allows it to escape from blood vessels into the extracellular spaces more

easily, where it participates in the defence of tissue fluid and body surfaces. Its primary functions are to facilitate the removal of microorganisms, neutralise toxins and to bind to microorganisms or infected cells. It can also lead to opsonisation and cause activation of the complement system.

Immunoglobulin M (IgM)

Immunoglobulin M is found in second highest concentration, forming up to 15% of the total immunoglobulins present. It exists either as a monomer or as a large polymer. The polymer form consists of five (occasionally six) 180 kDa units linked by disulphide bonds in a circular fashion, having a large molecular weight of 900 kDa. Similar to IgG, it is made and secreted by plasma cells in the spleen, lymph nodes and bone marrow.

Immunoglobulin M is the major immunoglobulin class produced during a primary immune response. It is also produced in a secondary response, but is usually masked by the predominance of IgG (Figure 56.3). Although produced in small quantities, IgM is considerably more efficient than the IgG on equimolar concentration at complement activation, opsonisation, neutralisation of virus and agglutination. However, because of its big size, IgM molecules are mainly confined to the blood vessels. As a result, they are probably of little importance in providing protection in body fluids or secretions, even at sites of acute inflammation.

Immunoglobulin E (IgE)

Immunoglobulin E is secreted by plasma cells mainly located in tissue just under body surfaces i.e. walls of intestinal tract, respiratory tract, urinary system and skin. It is typical Y-shaped immunoglobulin with two heavy and 2 light chains (Figure 56.6). The IgE has a molecular weight of about 190 kDa and is found in very low concentrations in serum. However, because of the low concentrations, it cannot act simply by binding and coating the antigens.

Immunoglobulin E acts mainly as a signal transducing molecule; therefore, it is normally found bound to receptors on mast cells and basophils. As discussed earlier (Chapter 46), cross linkage of two IgE molecules results in mast cell degranulation and subsequent release of a number of preformed (e.g. histamine and serotonin) and synthesised (e.g. eicosanoids) mediators. The resulting acute inflammation enhances local defences and helps eliminate the invading microorganisms. Immunoglobulin E plays a major role in the response to parasites and in pathogenesis of allergic diseases and mediates type I hypersensitivity reaction.

Immunoglobulin A (IgA)

Similar to IgE, the IgA is secreted by plasma cells located beneath the body surfaces. It has a molecular weight of about 150 kDa, but is mostly secreted as dimmer (higher polymers of IgA may also be occasionally found in serum). The IgA produced by body surfaces may either pass through epithelial cells into external secretions or diffuse into blood stream. Therefore, IgA is the predominant immunoglobulin found in body secretions such as saliva, milk, tears, intestinal fluid and respiratory secretions. This protects the intestine, respiratory tract, urinary tract, mammary glands, eyes, etc. against microbial invasion. IgA is also unique in that is can act inside cells. It can agglutinate particulate antigen and neutralise viruses, but it does not activate the complement system and cannot act as an opsonin.

Immunoglobulin D (IgD)

Immunoglobulin D is primarily a B-cell receptor (BCR) and is rarely encountered in body fluids. It is not found in all mammalian species. It is found mainly in humans, subhuman primates, rodents and probably in dogs. But it is not present in pigs and rabbits and probably in other domestic animals.

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http://en.wikipedia.org/wiki/Immune_system

http://en.wikipedia.org/wiki/Lymphocyte

CHAPTER 57 IMMUNOMODULATORS (BIOLOGICAL RESPONSE MODIFIERS)

- Immunosuppressants
  - General principles
  - Classification
  - Agents preventing activation of immune system
    - Antibiotics/Calcineurin inhibitors
    - Glucocorticoids
    - TNF-α inhibitors
    - IL-1 receptor antagonists
    - Miscellaneous drugs
  - Agents inhibiting proliferation and expansion of immune cells
    - Alkylating agents
    - Antimetabolites
    - Vinca alkaloids
    - Antibiotics
  - Agents interfering with antigen/Antibody presentation
    - Immunoglobulins/Antibodies
      - Polyclonal antibodies
      - Monoclonal antibodies
        - Anti-CD monoclonal antibodies
        - Anti-compliment component monoclonal antibodies
        - Anti-TNF monoclonal antibodies
        - Miscellaneous monoclonal antibodies
      - Fusion proteins
    - Androgenic steroids
- Immunostimulants
  - Classification
  - Immunostimulants of microbial origin
  - Immunostimulants of animal origin
    - Cytokines
      - Interleukins
      - Interferons
      - Colony Stimulating Factors
        - Granulocyte-colony stimulating factor
        - Granulocyte-macrophage colony-stimulating factor
        - Stem cell factor
    - Hormones
  - Immunostimulants of plant origin
  - Synthetic immunostimulants
  - Immunostimulants of natural origin
    - Vitamins
    - Minerals
  - Miscellaneous immunostimulants

Immunomodulators or Biological response modifiers are agents which regulate or modify the host's immune response to a microbe, neoplasm or inflammatory response. The term immunomodulator is a general term used for all substances which modulate or alter the immune system, whereas the term biological response modifier (BRM) is used mostly for substances which the human or animal body produce naturally in response to an infection or a neoplasm. However in general practice, both the terms are used interchangeably and carry the same meaning.

The area of pharmacology dealing with the drugs interfering with or modulating the immune response is comparatively new and is called 'immunopharmacology'. The major indications for pharmacological modulation of immune system in man and animals are prevention and treatment of autoimmune diseases and treatment of graft-versus-host reactions after organ transplantation. The goal of therapy may be either to decrease the effectiveness of immune response (immunosuppression) or to increase the effectiveness (immunostimulation). Accordingly, immunomodulators have been divided into two major classes - the immunosuppressants and immunostimulants.

IMMUNOSUPPRESSANTS

Immunosuppressants are a group of drugs which cause decrease in the magnitude of immune response. Immunosuppressants are used mainly to manage hypersensitivity reactions including autoimmune diseases and as anticancer drugs. A less common but increasingly growing use of immunosuppressants in small animals is in the organ transplantation.

General Principles

Several points should be kept in mind before recommending use of immunosuppressants in animals.

a. Primary immune response can be more easily and more effectively suppressed by drugs than the secondary (amnestic) response.

b. Immunosuppressants do not cause the same effect on all immune responses.

c. Inhibition of immune response is more successful if the immunosuppression therapy is begun before rather than after exposure to the inciting immunogen (antigen).

d. Care should be taken when using an immunosuppressant because patients receiving such drugs are predisposed to infections of any type.

Classification

Immunosuppressants can be broadly classified into following groups:

I. Agents preventing activation/initiation of immune system
   1. Antibiotics/Calcineurin inhibitors
      e.g. ciclosporin, tacrolimus and pimecrolimus.
   2. Glucocorticoids
      e.g. prednisolone.
   3. TNF-α inhibitors
      e.g. thalidomide and lenalidomide.
   4. Interleukin-1 receptor antagonists
      e.g. anakinra.
   5. Miscellaneous drugs
      e.g. abetimus and gusperimus.

II. Agents inhibiting proliferation and expansion of immune cells/Cytotoxic drugs
   1. Alkylating agents
      e.g. cyclophosphamide and chlorambucil.
   2. Antimetabolites
      e.g. azathioprine, methotrexate, mycophenolate mofetil and leflunomide.
   3. Vinca alkaloids
      e.g. vincristine and vinblastine.
   4. Antibiotics
      e.g. dactinomycin, sirolimus and everolimus.

III. Agents interfering with antibody/antigen presentation
   1. Immunoglobulins/Antibodies
   a. Polyclonal antibodies
      e.g. antithymocyte globulin, human immune globulin and anti-lymphocyte globulin.
   b. Monoclonal antibodies
   i. Anti-CD monoclonal antibodies
      e.g. muromonab CD3, efalizumab, basiliximab otelixizumab and rituximab.
   ii. Anti-compliment component monoclonal antibodies
      e.g. eculizumab.
   iii. Anti-TNF monoclonal antibodies
      e.g. infliximab, adalimumab, afelimomab and golimumab.
   iv. Miscellaneous monoclonal antibodies
      e.g. faralimomab, mepolizumab, natalizumab, daclizumab and tocilizumab.
   2. Fusion proteins
      e.g. etanercept, abatacept, belatacept, pegsunercept and rilonacept.
   3. Synthetic androgens
      e.g. danazol.

I. AGENTS PREVENTING ACTIVATION / INITIATION OF IMMUNE SYSTEM

1. Antibiotics/Calcineurin Inhibitors

Ciclosporin

Ciclosporin (Cyclosporin, cyclosporin A, cyclosporine) is a cyclic polypeptide obtained from the fungi *Cyclindrocarpon lucidium* and *Trichoderma polysporum*. It is very lipophilic drug, which must be solubilised before administration. The IV preparation is an ethanol polyoxyethylated castor oil mixture, whereas the oral preparation is a soft gelatine capsule or a newer microemulsion formulation. It is a highly selective immunosuppressant and is widely used in human medicine for the organ transplantation and treatment of selective autoimmune disorders.

Chemistry

Ciclosporin is a cyclic polypeptide with 11 amino acids. All amide nitrogen in the structure is either hydrogen bonded or methylated. Ciclosporin A is one of the nine ciclosporins (A through I), each a cyclic peptide drug.

Mechanism of action

Ciclosporin is more effective against T cell-dependent immune mechanisms such as those underlying transplant rejection and some forms of autoimmunity. Ciclosporin readily enters T lymphocytes and binds to a cytoplasmic receptor protein called cyclophilin (immunophilin). The cyclophilin-ciclosporin complex binds to and inhibits calcineurin, a calcium dependent phosphatase. Inhibition of calcineurin prevents dephosphorylation of regulatory cytoplasmic proteins which normally mediate movement of the transcription factor NFAT (nuclear factor of activated T cells) to the nucleus. The NFAT is normally required for transcription of interleukin 2 (IL-2) and other growth and differentiation associated cytokines (e.g. IL-4, GM-CSF and TNF-α). Failure of NFAT to enter the nucleus does not allow activation of gene transcription and subsequent cytokines production. Blockade of IL-2 and other cytokines lead to impaired cytotoxic and helper T cell proliferation (Figure 57.1). As a result, the T

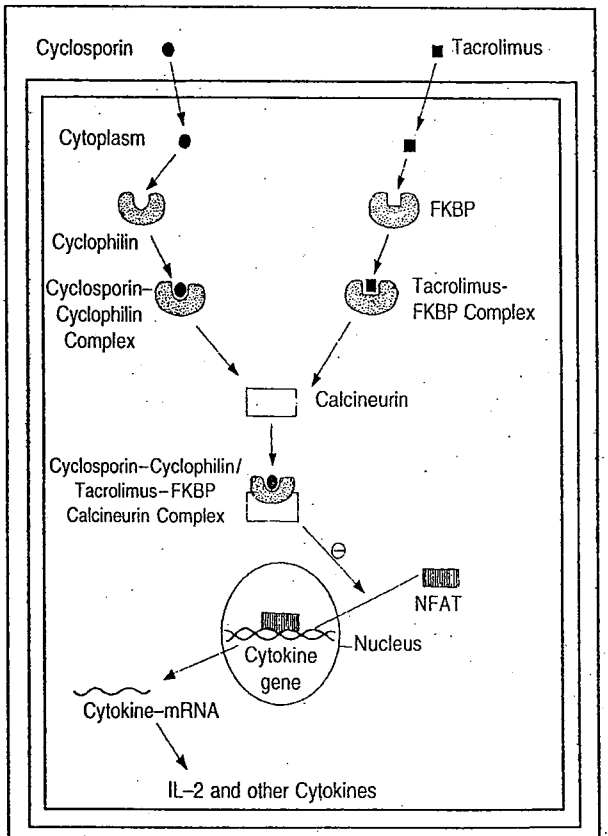


Fig. 57.1. Schematic representation of mechanism of action of cyclosporin and tacrolimus (see text for detail).
Abbreviations : FKBP = FK506-binding protein, NFAT = nuclear factor of activated T-lympocyte; mRNA = messenger ribonucleic acid; IL-2 = interleukin 2; – = inhibition.

lymphocytes fail to respond to specific antigenic stimulation. Inhibition of interferon-α (IFN-α) production also prevents activation of macrophages (IFN-α is an important signal for macrophage activation).

Ciclosporin has also been shown to increase expression of transforming growth factor β (TGF-β), a potent inhibitor of IL-2 stimulated T cell proliferation and generation of cytotoxic T lymphocytes. Ciclosporin is most active when administered before antigen exposure. However, it can suppress the responses of primed helper T cells also; therefore it is useful in autoimmune disease as well. The B cells are not affected by ciclosporin.

Pharmacological effects

The pharmacological effects of ciclosporin are complex. It selectively suppresses cell-mediated immunity without significantly affecting the humoral immunity. It specifically inhibits activation of helper T (Th) cells early in their immune response to antigenic and regulatory stimuli and suppresses production of many cytokines including IL-2 without any effect on suppressor T cells. It suppresses immune responses during organ transplantation of kidney, heart, bone marrow, lungs, etc. to prevent graft rejection. It also modulates immune-mediated destruction of tear secreting tissues and stimulates tear production. In addition, it acts on cornea to control the pigmentary keratitis that accompanies many case of keratoconjunctivitis sicca (KCS) that occurs in dogs.

Pharmacokinetics

The pharmacokinetic behaviour of ciclosporin has not been studied in detail in domestic animals. Absorption of ciclosporin after oral administration is quite variable and depends on several factors including type of formulation used and presence of fatty meal. In humans, oral bioavailability of gelatine capsule ranges from 20 to 50 percent and is enhanced by administration of a fatty meal. A modified microemulsion formulation gives a uniform and slightly increased bioavailability, but absorption of drug is not dependent on bile acids. After absorption, ciclosporin is widely distributed in body and accumulates in erythrocytes (about 50% of circulating drug) and leucocytes (about 10-20% of circulating drug). Remaining circulating drug is bound to plasma proteins.

Ciclosporin undergoes extensive biotransformation in liver and gives rise of a number of metabolites, which vary among animals. Metabolites are excreted in bile and faeces. The elimination half-life of ciclosporin in humans is about 6 hours.

Side effects/Adverse effects

Ciclosporin has a narrow therapeutic index in human patients, with renal toxicity as the primary adverse effect. It also impairs the liver function and produces hypertension. Ciclosporin increases the incidence of gallstones and sometimes lymphomas in human patients. The risk of renal and liver toxicities appears to be less in small animals. Side effects in dogs have included gastrointestinal disturbances, weight loss, gingival hyperplasia, shaking and hirsutism. Cats do not like the unpalatable taste of ciclosporin, but otherwise tolerate the drug well. The commercial form intended for IV use has been associated with anaphylactoid reactions in dogs due to presence of a solubilising agent.

Contraindications and precautions

Ciclosporin should not be used in immunocompromised patients. Its use should be avoided in pregnant and lactating animals. It should not be used in dogs less than 6 months of age or less than 2 kg body-weight. Ciclosporin concentrations should be monitored as a guide to effective yet safe dosing regimen. Trough concentrations of ciclosporin in blood should be maintained in the 250-400 ng/ml range. Topical application of ciclosporin in animals should be done carefully. Operators should wear impervious gloves when applying ointment and hands should be washed immediately after topical application of ciclosporin.

Drug interactions

Ciclosporin shows interactions with a large number of drugs. All nephrotoxic drugs like aminoglycosides, vancomycin and amphotericin B enhance its toxicity. By depressing renal function, ciclosporin can reduce excretion of many drugs. Microsomal enzyme inducers (e.g. phenobarbital and phenytoin) lower its blood concentration and increase chances of transplant rejection. On the other hand, erythromycin, ketoconazole, cimetidine and related drugs may inhibit hepatic drug-metabolising enzymes and enhance toxicity. Drugs like diltiazem, verapamil and itraconazole decrease clearance of ciclosporin. Potassium supplements and K^+ sparing diuretics can produce marked hyperkalaemia.

Clinical uses

Ciclosporin is used in both human and veterinary practices for organ transplantation and treatment of autoimmune diseases. In veterinary medicine, it is used to prevent kidney rejection after renal transplantation in cats and to treat perianal fistulas and keratoconjunctivitis sicca in dogs. Ciclosporin also is being used increasingly in patients suffering from autoimmune disorders (e.g. pure red cell aplasia) which have not responded to traditional immunosuppression therapy. Other indications for ciclosporin may include treatment of aplastic anaemia, inflammatory bowel disease and several dermatological diseases. The drug exhibits very poor solubility in water and, as a consequence, suspension and emulsion forms of the drug have been developed for oral administration and for injection. A newer microemulsion, orally-administered formulation is available as a solution and as soft gelatine capsules. It is available as an ophthalmic ointment for dogs.

Dose

Perianal fistulas

Dogs : 5 – 10 mg/kg, PO, 2 times daily. Dose should be adjusted based on monitoring of the drug concentration.

Renal allografts

Dogs & cats : 7.5 mg/kg, PO, 2 times daily coupled with prednisolone (0.125-0.25 mg/kg, PO, 2 times daily). Dose of ciclosporin should be adjusted based on monitoring of the drug concentration.

Keratoconjunctivitis sicca

Ophthalmic ointment (0.2%)

Dogs : Ophthalmic application, 2 times daily

Ophthalmic solution (1% solution in oil)

Dogs : One drop in eye, 2 times daily.

Other autoimmune disorders

Dogs & cats : 5 – 10 mg/kg; PO, 2 times daily.

: 4 – 6 mg/kg as a 4 hour IV infusion (IV infusion is recommended for animals in which oral administration is not possible).

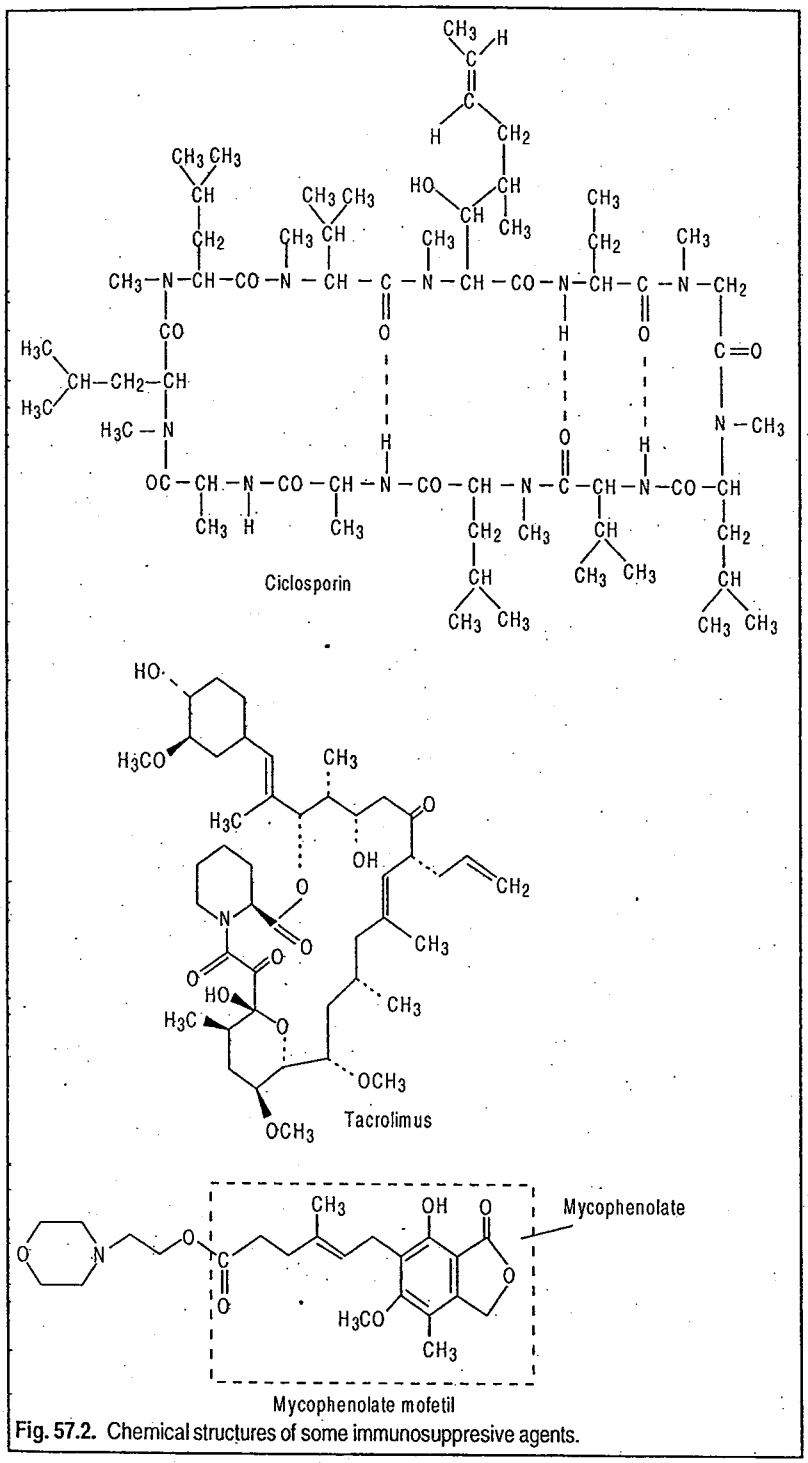


Fig. 57.2. Chemical structures of some immunosuppresive agents.

Tacrolimus

Tacrolimus (Fk-506 or Fujimycin) is a 23-member macrolide lactone antibiotic obtained from fungus *Streptomyces tsukubaensis*. Although tacrolimus is chemically different from ciclosporin, but both drugs share a similar mechanism of action. Similar to ciclosporin, tacrolimus rapidly diffuses into the cytoplasm of T cell. However, it binds to a different intracellular receptor labelled as Fk506-binding protein-12 (FkBP-12). The tacrolimus-FkBP-12 complex then binds to and inhibits calcineurin (Figure 57.1). As described for ciclosporin, the inhibition of calcineurin phosphatase activity prevents dephosphorylation and nuclear translocation of NFAT thus inhibiting both T-lymphocyte signal transduction and IL-2 transcription. Thus it inhibits, directly or indirectly, the *de novo* expression of nuclear regulatory proteins and T cell activity genes. Tacrolimus *in vitro* is much more potent an inhibitor of lymphocyte activation than is ciclosporin. It also inhibits B cell proliferation and production of antibody by unknown mechanism(s). The pharmacological properties of tacrolimus are similar to those of ciclosporin, but it is about 100 times more potent.

Tacrolimus is administered orally as well as by IV infusion. It exhibits a longer half-life (12-24 hours) and has the same therapeutic application as well as toxicity profile in humans. The drug is considered much more toxic than ciclosporin in dogs. The side effects include anorexia, vasculitis, cardiac damage, hypertension, blurred vision, and liver and kidney problems. It may also potentially increase the severity of existing fungal or viral infections. Combination therapy with ciclosporin appears to have an additive effect with less toxicity. As an ointment, tacrolimus is used in the treatment of eczema, particularly atopic dermatitis. It suppresses inflammation in a similar way to steroids and is equally as effective as a mid-potency steroid.

Pimecrolimus

Pimecrolimus is an immunomodulating agent mainly used in the treatment of atopic dermatitis (eczema). Pimecrolimus, like tacrolimus, inhibits T-cell activation by the calcineurin pathway and decreases release of numerous cytokines, thereby preventing the cascade of immune signals. Pimecrolimus also prevents the release of inflammatory cytokines and mediators from mast cells. It is currently available as a topical cream.

2. Glucocorticoids

Glucocorticoids are considered first-line therapy for most immune mediated disorders in veterinary patients. They have potent immunosuppressant and anti-inflammatory actions. They have more marked effect on cell-mediated immunity. They particularly inhibit MHC expression, and activation and proliferation of T-lymphocytes. Expression of several cytokines including IL-1, IL-2, IL-6, TBF-α and INF-α is depressed and T-cell dependent immunity is impaired by use of glucocorticoids.

Mechanism of action

Glucocorticoids have actions at a variety of target cells. Glucocorticoids after absorption bind to a specific glucocorticoid receptor in the cell cytoplasm. The glucocorticoid-receptor complex is then transported to cell nucleus (see for detail Chapter 51), where it stimulates synthesis of a protein called IkB. The IkB then binds to (forms complex) and suppresses activities of a transcription factor called NFkB. This leads to suppression of gene encoding cytokines. Many genes encoding cytokines also

have a glucocorticoid response element in the 5' regulatory sequence that is target for the complex formed by the association of glucocorticoid with its intracellular receptor protein.

Glucocorticoids also contribute to immunosuppression through their non-specific anti-inflammatory effects. In the presence of glucocorticoids, neutrophils and monocytes display poor chemotaxis and decreased lysosomal enzyme release. Glucocorticoids also inhibit increase in vascular permeability and vasodilatation; as a result they prevent oedema formation and fibrin deposition. Glucocorticoids also lyse and possibly induce redistribution of lymphocytes causing a rapid and transient decrease in peripheral lymphocytic blood count.

The most commonly used glucocorticoids for immune disorders are prednisone and prednisolone. These drugs are required for long-term and in large doses to prevent transplant rejection, particularly bone marrow transplant. They are also used in autoimmune disorders and to prevent allergy arising from use of antilymphocytic globulin or monoclonal antibodies. Glucocorticoids are used either alone or in combination with other immunosuppressive agents. Increased risk of infections, ulcers, hyperglycaemia and osteoporosis are the main adverse effects of glucocorticoids. The chemistry, pharmacological effects, pharmacokinetics, drug interactions, dose, etc. of glucocorticoid agents have been described in detail in Chapter 51.

3. TNF-α Inhibitors

Tumour necrosis factor (TNF) is a member of a group of cytokines involved in systemic inflammation and regulation of immune cells. Dysregulation of TNF production has been implicated in a variety of human diseases including inflammatory bowel disease (IBD) and rheumatoid arthritis: Inhibition of TNF is a novel approach to treat disorders associated with enhanced TNF production.

Thalidomide

Thalidomide is a drug of historical importance that produced thousands of malformed babies in Europe in the late 1950's and was later on withdrawn from market in early 1960's. Recently, clinicians have again shown interest in this drug due to its immunomodulatory, anti-inflammatory and antiangiogenesis effects. Currently it is being used in human medicine, combined with dexamethasone, primarily to treat multiple myeloma. Thalidomide is also being approved for the treatment of erythema nodosum leprosum, a painful skin condition. Thalidomide also inhibits the growth of new blood vessels (angiogenesis), which may be useful in treating macular degeneration and other diseases. There are studies underway to determine its efficacy in some kinds of cancers. The primary mechanism of immunomodulatory action of thalidomide is considered to be due to its antagonistic effect on TNFα. Thalidomide is not recommended in pregnancy due to its potential teratogenic effects. Main side effects as reported in human patients are drowsiness, constipation, rash, numbness, tingling, dryness of mouth and migraine.

Lenalidomide

Lenalidomide is a structural analogue of thalidomide. It was initially introduced for the treatment of multiple myeloma, but has now shown efficacy in a number of haematological and inflammatory disorders, and cancers. Potential side effects of lenalidomide are thrombosis, pulmonary embolus and hepatotoxicity, as well as bone marrow toxicity resulting in neutropenia and thrombocytopenia. Myelosuppression is also a major dose-limiting adverse effect.

4. Interleukin-1 Receptor Antagonists

Anakinra

Anakinra is an interleukin-1 (IL-1) receptor antagonist synthesized artificially from cultures of genetically modified *Escherichia coli* using recombinant DNA technology. It differs from native human IL-1RA in that it has addition of a single methionine residue on its amino terminus. Anakinra acts by competitively blocking the binding of IL-1 to the interleukin-1 type receptor. As discussed earlier, IL-1 is expressed in many tissues and organs in response to inflammatory and immunological responses.

Anakinra is indicated for the management of signs and symptoms of rheumatoid arthritis and to inhibit the progression of structural damage associated with the disease. In rheumatoid arthritis, excessive IL-1 activity has been associated with inflammation and cartilage degradation as a result of loss of proteoglycans. IL-1 additionally stimulates bone resorption in rheumatoid arthritis. Blockade of IL-1 activity by anakinra, therefore, produces desirable anti-inflammatory and immunosuppressive activity in patients with rheumatoid arthritis. In rheumatoid arthritis, it is used as monotherapeutic agent or in combination with a 'Disease-modifying antirheumatic drug (DMARD). Its use in combination with anti-TNF agents such as etanercept, infliximab or adalimumab is not recommended. Gastrointestinal upsets (nausea, diarrhoea and abdominal pain), allergic reactions, respiratory signs, blood disorders and pain at injection site are some important side/adverse effects.

5. Miscellaneous Drugs

Abetimus

Abetimus is a synthetic immunosuppressant belonging to a novel class of drugs called tolerogens, the group of drugs which induce tolerance and make the tissue non-responsive to antigens. Abetimus is made up of four double-stranded oligodeoxyribonucleotides those are attached to a carrier platform and are designed to block specific B-cell anti double stranded DNA antibodies. It may also complex anti double stranded DNA antibodies together, therefore disabling them. Abetimus sodium has been designed to treat Systemic Lupus Erythematosus.

Gusperimus

Gusperimus is a derivative of the antitumor antibiotic spergualin. It produces immunosuppressive actions by inhibiting the interleukin-2-stimulated maturation of T cells to the S and G2/M phases. It also results in the inhibition of growth of activated naive CD4 T cells. It is classified as orphan drug and is being used for treatment of Wegener's granulomatosis, a serious form of vasculitis frequently associated with permanent disability and/or fatal outcome.

II. AGENTS INHIBITING PROLIFERATION AND EXPANSION OF IMMUNE CELLS

Drugs which inhibit proliferation and expansion of immune cells include cytotoxic drugs used in the treatment of cancer. Most of these drugs are potent and potentially dangerous, so extreme care is required in their use. Cytotoxic drugs are not selective in their action on growing and dividing cells hence organs which contain populations of rapidly dividing cells (e.g.

bone marrow and gastrointestinal tract) are particularly susceptible to their toxic effects. Cytotoxic drugs have been discussed in detail in Chapter 72.

1. Alkylating Agents

Cyclophosphamide

Cyclophosphamide is a nitrogen-mustard derivative alkylating agent that is widely used as antineoplastic and immunosuppressive drug. It has more marked effect on B cells and humoral immunity compared to that on T cells and cell mediated immunity.

The immunomodulatory effect of cyclophosphamide reflects the same mechanism of action as its anticancer effects. Cyclophosphamide is converted to active metabolites (e.g. phosphoramide) in body, which alkylates DNA in both proliferating and non-proliferating cells; proliferating cells are more susceptible to alkylation (see for detail Chapter 72). Although it impairs both B and T cells, the immune response of B cell is greatly affected because its rate of recovery is much slower (thus mainly suppressing humoral immunity). Cyclophosphamide also prevents B cells to renew their antigen receptors.

Cyclophosphamide is used as an immunosuppressant primarily due to its immunosuppressive effects on lymphoid elements in bone morrow transplantation. In other organ transplants, it is employed only as a reserve drug. It is also used to treat some autoimmune disorders like systemic lupus erythematosus, granulomatosis and idiopathic thrombocytopenia purpurea. Toxicity of cyclophosphamide is typical of drugs which target proliferative cells (e.g. pancytopenia and cardiotoxicity). In addition, haemorrhagic cystitis has been reported in dogs receiving the drug. The pharmacology of cyclophosphamide has been described in Chapter 72.

Chlorambucil

Chlorambucil is also a nitrogen mustard-alkylating agent possessing both antineoplastic and immunosuppressive effects. Similar to cyclophosphamide, chlorambucil acts as a prodrug that is metabolised in liver to its active form phenylacetic acid. Its cytotoxic effects are similar to those of cyclophosphamide; however, it has relatively weak immunosuppressant action. Its effect is slow and gradual in onset.

Chlorambucil is sometimes utilised in autoimmune diseases and transplant maintenance regimens. It may also be useful as adjunctive therapy for some immune-mediated conditions like glomerulonephritis, non-erosive arthritis, or immune-mediated skin diseases. In cats, it has been recommended for routine treatment for feline pemphigus foliaceus and for severe recalcitrant cases of feline eosinophilic granuloma complex. Toxicities are uncommon but may include mild, gradual and rapidly reversible bone marrow suppression. Anorexia, vomiting and diarrhoea have also been reported but may resolve when the drug is changed from a daily to an alternate day schedule.

Dose

Cats : 0.1 – 0.2 mg/kg, PO, once daily initially, then every alternate day.

2. Antimetabolites

Azathioprine

Azathioprine is a purine antimetabolite and a pro-drug of 6-mercaptopurine. It has more marked immunosuppressive effect than the antineoplastic effect. In veterinary practice, it is used mainly in the treatment of immune-mediated diseases which cannot be adequately controlled with glucocorticoids alone.

Mechanism of action

Azathioprine is a prodrug that contains the 6-mercaptopurine moiety. The 6-mercaptopurine is the thiol analogue of hypoxanthine. In body, azathioprine undergoes a slow activation by nucleophiles such as glutathione to form the active drug 6-mercaptopurine (6-MP). The 6-mercaptopurine then undergoes further transformation to the corresponding nucleotide 6-mercaptopurine ribose phosphate (6-MPRP) better known as

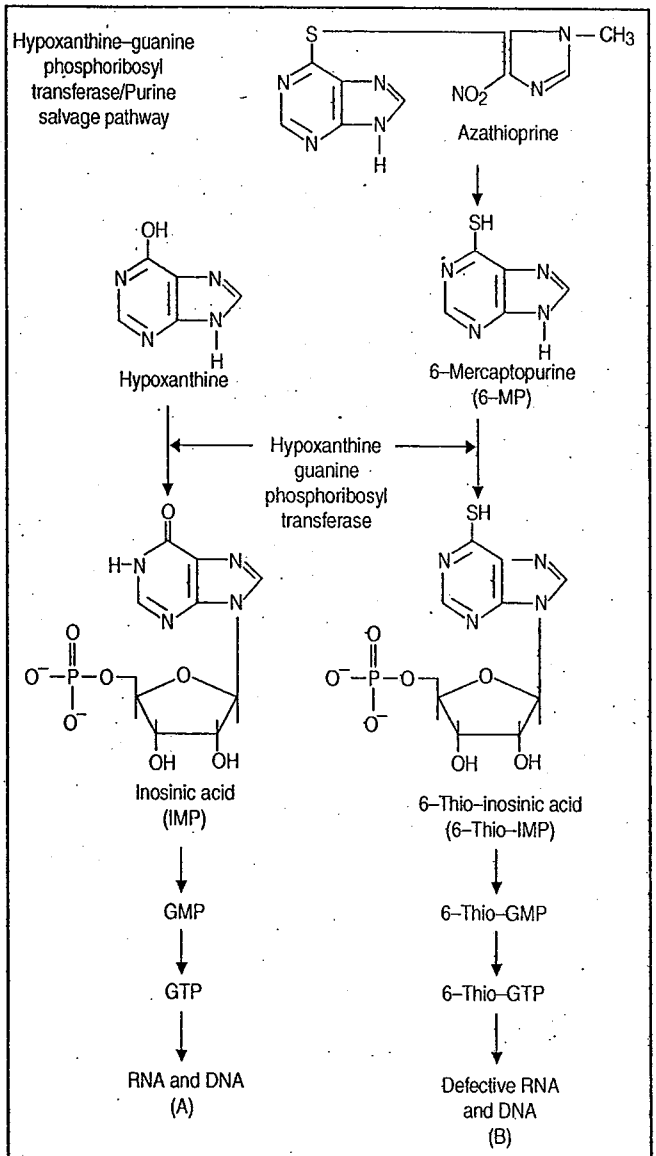


Fig. 57.3. Proposed mechanism of action of azathioprine and 6-mercaptopurine (see text for detail).
A : Normal synthesis of RNA and DNA.
B : Defective synthesis of RNA and DNA.
Abbreviations : GMP = guanosine monophosphate ; GTP = guanosine triphosphate ; RNA = ribonucleic acid, DNA deoxyribonucleic acid.

6-thioinosic acid (6-thio-IMP). The 6-thio-IMP is converted to 6-thio-GMP and finally to 6-thio-GTP, which is incorporated into RNA. The deoxyribonucleotide analogues, which are also formed, are incorporated into the DNA. This causes damage to DNA and inhibition of gene transcription (Figure 57.3), thus impeding the proliferation of cells, especially the fast-growing lymphocytes. T-cells and B-cells are particularly affected by the inhibition of purine synthesis.

Pharmacological effect

Azathioprine selectively affects differentiation, proliferation and function of T cells. It appears to be more potent immunosuppressive agent than does 6-mercaptopurine, which possesses a potent antineoplastic action. The basis of this difference is not clear, but may be due to the selective uptake of azathioprine into immune cells and its intracellular conversion to the active metabolite 6-MP. Azathioprine has greater activity on delayed hypersensitivity and cellular immunity than on humoral antibody responses. It also decreases natural killer cell population. Clinical response to azathioprine may require up to 6 weeks.

Pharmacokinetics

Azathioprine is well absorbed from the GI tract and is rapidly metabolised to 6-mercaptopurine, which is then metabolised to several other compounds. The half-life of azathioprine is about 10 minutes in humans and that of mercaptopurine is about 1 hour. Both azathioprine and mercaptopurine are moderately bound to plasma proteins. The metabolites of azathioprine are excreted in the urine.

Side effect/Adverse effects

Adverse effects of azathioprine mainly result from inhibited growth of rapidly growing cells including cells of bone marrow and GI tract. Accordingly side effects of azathioprine therapy include reversible bone marrow suppression (leucopenia, anaemia and thrombocytopenia) and GI toxicity. Cats are more prone to the bone marrow suppression, so azathioprine is not normally recommended in this species. Acute pancreatitis and hepatotoxicity have been reported in dogs. Other important adverse effects of azathioprine include increased susceptibility to infections or neoplasms on long-term use.

Contraindications and precautions

Azathioprine is contraindicated in patients hypersensitive to it. It is also not recommended during or within 2 weeks of vaccination. It should be used cautiously in patients with hepatic dysfunction. It should be withdrawn slowly because rapid withdrawal can lead to a rebound hyperimmune response. Azathioprine is mutagenic and teratogenic in laboratory animals.

Drug interactions

The hepatic metabolism of azathioprine may be decreased by concomitant use of allopurinol. Allopurinol blocks xanthine oxidase, an enzyme involved in the metabolism of metabolites of azathioprine. Azathioprine may inhibit or block the activity of neuromuscular blocking drugs (e.g. tubocurarine and pancuronium).

Clinical uses

In veterinary medicine, azathioprine is used mainly in the treatment of immune-mediated diseases which do not respond to glucocorticoid therapy alone. It is often combined with ciclosporin or prednisolone to prevent transplant rejection and for treatment of autoimmune disorders. In human medicine, azathioprine is used to prevent organ rejection following organ transplantation and to treat a vast array of autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, autoimmune hepatitis, atopic dermatitis and myasthenia gravis. Complete blood count and liver function tests should be monitored during therapy.

Dose

Dogs : 1 – 2 mg/kg, PO, every 1 to 2 days.

Cats : 0.2 – 0.3 mg/kg, PO, every 1 to 2 days.

Note: Some practitioners do not recommend azathioprine for use in cats due to severe toxicity and difficulty in accurately dosing.

Methotrexate

Methotrexate is a folic acid antagonist that is primarily used as an antineoplastic agent. It also possesses immunosuppressant and anti-inflammatory actions. It depresses cytokine production and cell mediated immune response. As an immunosuppressant, it is mainly used in human medicine for many autoimmune diseases like rapidly progressive rheumatoid arthritis, myasthenia gravis and uveitis. Its use as immunosuppressant is not generally advisable in veterinary medicine because of the resultant toxic effects. Myelosuppression and gastrointestinal ulcerations are common adverse effects observed in small animals.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an immunosuppressant that inhibits lymphocyte proliferation, antibody production and cell-mediated immunity. Unlike other anticancer drugs, MMF and its active metabolite are relatively selective for lymphocytes.

Mycophenolate mofetil is a prodrug and an ester of mycophenolic acid (MPA). In the body, it is rapidly metabolised to the active drug mycophenolic acid. Mycophenolic acid selectively inhibits inosine monophosphate dehydrogenase, an enzyme essential for *de novo* synthesis of guanosine nucleotide in the T and B cells. The drug is highly selective for lymphocytes because T and B cells solely rely on *de novo* purine biosynthesis, while other cell types can use purine salvage pathway (hypoxanthine-guanine phosphatidyl transferase pathway). Mycophenolate mofetil, therefore, selectively inhibits lymphocyte proliferation and functions including antibody formation, cellular adhesion and migration, without affecting bone marrow and parenchymal cells.

Mycophenolate mofetil is presently approved for prevention of renal allograft rejection in human patients. It is used mostly in combination with glucocorticoids and ciclosporin. It helps to reduce the dose of ciclosporin and thus its toxicity. The drug currently is under investigation for application in canine immune-mediated disorders.

Leflunomide

Leflunomide is an immunosuppressant drug that inhibits *de novo* biosynthesis of pyrimidine by blocking dihydroorotate dehydrogenase enzyme. At high concentrations, leflunomide is also an inhibitor of tyrosine kinases associated with growth factor receptors. Following oral administration, leflunomide is metabolized to teriflunomide, which is responsible for all of the drug's activity *in vivo*. Leflunomide is indicated for treatment moderate to severe rheumatoid arthritis and psoriatic arthritis in humans. Like MMF, leflunomide currently is under study for use in animals.

3. Vinca Alkaloids

Vincristine

Vincristine is a vinca alkaloid used widely as an antineoplastic drug. Sometimes it is also used to treat immune-mediated thrombocytopenia because of its ability to cause maturation and release of mature thrombocytes from the bone marrow by stimulation of megakaryocyte endomitosis. In veterinary medicine, vincristine as an immunosuppressant is recommended only when other therapies (e.g. glucocorticoids) are ineffective and bone marrow aspirate demonstrates adequate megakaryocytopoiesis. Vincristine is less myelosuppressive at usual doses, but may cause peripheral neurotoxic effects. The detailed pharmacology of vincristine has been given in Chapter 72.

Dose

For immune-mediated thrombocytopenia

Dogs : 0.01 – 0.025 mg/kg, IV, once every 7 – 10 days. Maximum dose should not exceed 1.5 mg (total).

Vinblastine

Vinblastine is another vinca alkaloid used mainly as antineoplastic drug. It has also been used to treat Type II hypersensitivity reactions, especially immune-mediated haemolytic anaemia (IMHA). It appears to decrease expression of Fc (IgG) receptors on macrophages. Unlike vincristine, vinblastine causes myelosuppression and may induce severe perivascular reactions.

Dose

Dogs & cats : 0.1 – 0.4 mg/kg, IV, once every 7 -14 days.

4. Antibiotics

Dactinomycin

Dactinomycin (Actinomycin D) is a potent antineoplastic drug. It also possesses immunosuppressing activity. Exact mechanism of dactinomycin for its immunosuppressive activity has not been determined, but it apparently binds with double-helical DNA and interferes with DNA's template activity. Dactinomycin has not been extensively used in veterinary medicine for immunomodulation. Important adverse effects of dactinomycin include anaemia, leucopenia, thrombocytopenia, vomiting, GI ulcerations and diarrhoea due to the selective damage to haematopoietic and intestinal tissues.

Sirolimus

Sirolimus (Rapamycin) is a macrocyclic lactone (macrolide antibiotic) produced by *Streptomyces hygroscopicus*. It is similar in structure to tacrolimus (FK 506) and forms a complex with intracellular protein FkBP-12. However, it does not affect calcineurin activity, but binds to and inhibits mammalian kinase, a key enzyme in the cell-cycle proliferation.

Sirolimus has been recommended for use in human medicine for prophylaxis of organ transplant rejection. It is used in combination therapy with a calcineurin inhibitor (ciclosporin or tacrolimus) and glucocorticoids. Important adverse effects of sirolimus include anaemia, leucopenia, thrombocytopenia, fever, and GI effects. Studies are underway to evaluate the clinical use of sirolimus in small animals.

Everolimus

Everolimus is the 40-O-(2-Hydroxyethyl) derivative of sirolimus and works similarly to sirolimus. It is currently used as an immunosuppressant to prevent rejection of organ transplants.

III. AGENTS INTERFERING WITH ANTIGEN / ANTIBODY PRESENTATION

1. Immunoglobulins/Antibodies

Both polyclonal and monoclonal antibodies against lymphocyte cell surface antigens are widely used in human medicine for prevention and treatment of organ transplant rejection. They provide rapid reduction in the number of lymphoid cells and suppress the function of specific lymphocytes. The clinical use of these agents in veterinary medicine is limited.

a. Polyclonal Antibodies

Polyclonal antibodies are a heterogeneous population of immunoglobulin molecules secreted against a specific antigen, each identifying a different epitope (antigenic determinant part). They are generated mainly by repeated injection of thymocytes or lymphocytes into a variety of animals such as horses, rabbits, goats, sheep or chickens and then purifying the serum immunoglobulins fraction. Although highly effective and less expensive, they vary in efficacy and toxicity from batch to batch.

Antithymocyte globulin

Antithymocyte globulin (ATG) or antilymphocyte globulin (ALG) is a purified gamma globulin prepared from horse, rabbit or other animals immunised with human thymocytes (thymic lymphocytes). It contains multiple cytotoxic antibodies which bind to several antigens (e.g. CD2, CD3, CD4, CD8, CD18, CD25, CD44, CD45, and HLA class I and II molecules) on the surface of human T lymphocytes. These antibodies deplete circulating lymphocytes by direct cytotoxicity (both complement and cell-mediated) and block lymphocyte function by binding to cell surface molecules involved in the regulation of cell function.

Antithymocyte globulin has been used primarily to suppress allograft rejection, especially in glucocorticoid resistant cases. ATG use can induce cytokine release syndrome, characterised by high grade fevers, chills and possibly rigors during administration. ATG has also been thought to increase the risk of post-transplant lymphoproliferative disorder. The other major side effects of ATG in human patients are serum sickness, glomerulonephritis and haematological complications (e.g. leucopenia and thrombocytopenia). Some of the adverse reactions can often be minimised by slowing the infusion rate. Two antithymocyte globulin (ATG) agents licensed for clinical use are Thymoglobulin (rabbit ATG, rATG) and Atgam (equine ATG, eATG).

Human immune globulin

Human immune globulin (Immune globulin human) or Human intravenous immunoglobulins (hIVIG) is a preparation of polyspecific immunoglobulins (IgG) that is obtained from the plasma of healthy human blood donors. Although it was first developed for the treatment of primary immunodeficiencies, it was soon discovered to have efficacy of treating immune mediated disorders. Although the exact mechanism of action is unknown, it may involve blockage of Fc receptors on macrophages leading to a decrease in B lymphocyte autoantibody production. It may also modulate antibody responses and cause IgG catabolism. Human immune globulin has been used with some success in dogs for treating immune-mediated thrombocytopenia and haemolytic anaemia. However, to avoid any allergic reaction to human proteins, its use has been limited to single IV dose treatment in dogs. During treatment, animal must be watched carefully for signs of anaphylactoid and other adverse reactions.

Anti-lymphocyte globulin

Anti-lymphocyte globulin (ALG) is an infusion of horse-derived or more commonly rabbit-derived antibodies against human T cells. It is used in the treatment of acute rejection in organ transplantation, especially in kidney transplants. It has also been used in the treatment of aplastic anaemia. It is less commonly used than the similar anti-thymocyte globulin (ATG), and like ATG it is associated with cytokine release syndrome in the short term and an increased risk of post-transplant lymphoproliferative disorder in the long term.

b. Monoclonal Antibodies

Monoclonal antibodies are a homogeneous population of antibodies which react only to a single epitope of defined specificity on the antigen. In contrast to the polyclonal antibodies, monoclonal antibodies are extremely efficient for binding of antigen within a mixture of related molecules and produce a more consistent and predictable immunosuppression, but they are more limited in their target specificity. Enzymes, bacterial toxins, radionuclides and cytotoxic drugs can now be linked to monoclonal antibodies making specific targeting of particular tissues or cell types possible.

i. Anti-CD Monoclonal Antibodies

The CD (Cluster of Differentiation) molecules are protein complexes composed of distinct chains. Because the CD molecules are involved in T cell activation and immunomodulation, drugs (often monoclonal antibodies) which target them are being investigated as immunosuppressant therapies.

Muromonab CD3

Muromonab CD3 (Muromonab CD3 monoclonal antibody) is a mouse monoclonal antibody against CD3 glycoprotein located on the surface of human T lymphocytes. The CD3 glycoprotein on T lymphocyte cells is located in close proximity to the antigen recognition receptor. Normally, the T cell receptor-CD3 complex transduces the signal for the T cell to proliferate, identify and attack the antigen. Binding of muromonab CD3 to the T cell receptor-CD3-complex on the surface of circulating T cells, initially leads to activation, but subsequently induces blockage and apoptosis of the T cells. As a result, participation of T cells in the immune response is prevented and T cell rapidly disappears from circulation and peripheral lymphoid organs such as lymph nodes and spleen. This protects the transplant against the T cells. The absence of detectable T cells from usual lymphoid region in such cases is secondary both to cell death (both complement and cell-mediated) and redistribution of T cells into non-lymphoid organs such as lungs. Muromonab CD3 also induces a reduction in function of the remaining T cells leading to an immune blocked state.

Muromonab CD3 is indicated for treatment of acute transplant rejection in human patients. It produces very rapid effect with circulating T cell disappearing from blood within minutes of drug administration; the T cells appear in circulation within approximately one week after termination of therapy. The response of this monoclonal antibody is less variable than to the polyclonal antithymocyte globulin (ATG). It is also less likely to produce allergic reactions, but is more expensive than ATG.

Important adverse effects of muromonab CD3 may include cytokine release syndrome with flu like symptoms. Occasionally, CNS toxicity (e.g. seizures, encephalopathy, oedema and aseptic meningitis), pulmonary oedema and anaphylactic reactions may be observed. High dose glucocorticoid pre-treatment prevents the release of cytokines and reduces other reactions.

Efalizumab

Efalizumab a recombinant humanized monoclonal antibody. It has a molecular weight of approximately 150 kDa and is produced in a Chinese hamster ovary mammalian cell expression system. Efalizumab binds to the CD11a subunit of lymphocyte function-associated antigen 1 and acts as an immunosuppressant. It acts to inhibit white blood cell migration out of blood vessels into tissues. Important side/adverse effects associated with efalizumab include bacterial sepsis, viral meningitis invasive fungal disease and progressive multifocal leukoencephalopathy (PML), a brain infection caused by reactivation of latent JC virus infection. Due to its association with some fatal brain infections, efalizumab has currently been withdrawn from the market.

Basiliximab

Basiliximab is a chimeric mouse-human monoclonal antibody to the α chain (CD25) of the IL-2 receptor of T cells. Antagonism at the interleukin-2 (IL-2) binding site on the surface of the activated T lymphocytes prevents T cells from replication and also from activating the B cells. This results in decreased production of antibodies in response to the transplant, thus protecting the transplant against the immune response. Basiliximab is used to prevent rejection in organ transplantation, especially in kidney transplants.

Other anti-CD monoclonal antibodies

A large number of other monoclonal antibodies against specific CD molecules are either available for clinical use or in advanced phases of clinical trials. These include monoclonal antibodies otelixizumab, teplizumab and visilizumab against CD3 molecule, clenoliximab, keliximab, zanolimumab against CD4 molecule, erlizumab against CD18 molecule, afutuzumab, ocrelizumab, pascolizumab, rituximab against CD20 molecule, lumiliximab against CD23 molecule, teneliximab and toralizumab against CD40 molecule, galiximab against CD80, gavilimomab against CD147 and ruplizumab against CD154 molecule.

ii. Anti-Compliment Component Monoclonal Antibodies

Eculizumab

Eculizumab is a monoclonal antibody directed against the complement protein C5. Eculizumab exhibits its therapeutic activity by binding to the C5 complement protein, thereby blocking its cleavage by the C5 convertase. This leads to decreased complement mediated activities like chemotaxis and immunosuppression. Eculizumab has been used in human patients for the treatment of paroxysmal nocturnal haemoglobinuria (PNH), a rare, acquired, potentially life-threatening disease of the blood characterized by complement-induced intravascular haemolytic anaemia, haemoglobinuria and thrombosis. Use of eculizumab reduces the need for blood transfusions, improves quality of life and reduces the risk of thrombosis. As eculizumab inhibits the complement activation and weakens the body defence system, it makes patients vulnerable to infection with encapsulated organisms.

iii. Anti-TNF Monoclonal Antibodies

Infliximab

Infliximab is a monoclonal antibody against tumour necrosis factor alpha (TNFα). As discussed earlier in Chapter 58, the TNFα

is a chemical messenger (cytokine) and a key part of the autoimmune reaction. TNF is primarily involved in the regulation of immune cells and is able to induce apoptotic cell death. It is also involved in the inflammatory processes. Infliximab neutralizes the biological activity of TNFα by binding with high affinity to the soluble (free floating in the blood) and transmembrane (located on the outer membranes of T cells and similar immune cells) forms of TNFα and inhibits or prevents the effective binding of TNFα with its receptors. Infliximab has been used in human medicine for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis and ulcerative colitis. Important side/adverse effects of the drug include serious and sometimes fatal blood disorders (leukopenia, neutropenia, thrombocytopenia, and pancytopenia), serious infections, chances of lymphoma and solid tissue cancers and hepatotoxicity. Similar to other TNF inhibitors, infliximab is an expensive drug.

Other drugs

Other anti-TNF monoclonal antibodies include adalimumab, afelimomab, certolizumab and golimumab. These drugs are largely used as immunosuppressants in human medicine for treatment of various immune mediated conditions including Crohn's disease and rheumatoid arthritis.

iv. Miscellaneous Monoclonal Antibodies

A large number of monoclonal antibodies have been synthesised with activity directed against interferons (e.g. faralimomab), interleukins (e.g. mepolizumab, elsilimomab and lebrikizumab), integrin (e.g. natalizumab) and interleukin receptors (e.g. daclizumab and tocilizumab). Several other monoclonal antibodies including abciximab, alemtuzumab, bevacizumab, cetuximab, daclizumab, gemtuzumab, natalizumab, nimotuzumab, omalizumab, palivizumab, panitumumab, ranibizumab, tositumomab and trastuzumab have also been developed and are either in advanced stages of clinical trials or available for clinical use in human practice for various anti-inflammatory, immunosuppressive and anti-neoplastic conditions.

3. Fusion Proteins

Fusion proteins or Chimeric proteins are proteins produced through joining of two or more genes which originally coded for separate proteins. Translation of this fusion gene results in a single polypeptide with functional properties derived from each of the original proteins. Recombinant fusion proteins are created artificially by recombinant DNA technology for use in biological research. Some fusion proteins have been developed as pharmaceutical agents and are being used for immunosuppressive purposes.

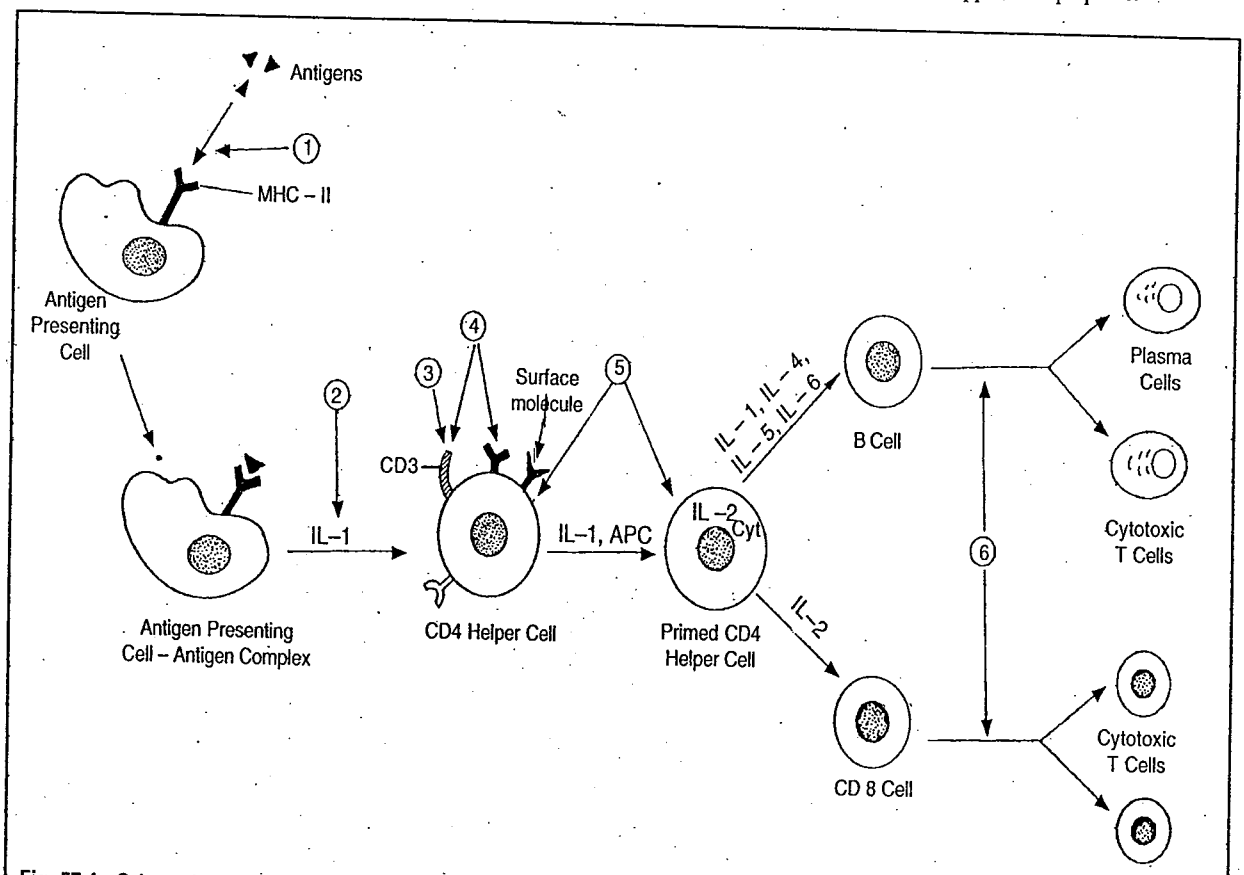


Fig. 57.4. Schematic representation of proposed sites of action of various immunosuppresive drugs (see text for detail).

1. Human immune globulin and danazol inhibit antigen recognition.
2. Glucocorticoids inhibit stimulation of IL-1.
3. Muromonab CD3 blocks CD3 glycoprotein on surface of T-lymphocytes.
4. Antithymocyte globulin inhibits various surface molecules (e.g. CD2, CD3, CD4) on T-lymphocyte.
5. Calcineurin inhibitors (e.g. ciclosporine, tacrolimus) and glucocorticoids inhibit expression of IL-2 and other cytokines and suppress activation and proliferation of T lymphocytes.
6. Cytotoxic drugs (e.g. azathioprine, cyclophosphamide) inhibit proliferation and differentiation of T and B lymphocytes.

Abbreviations : MHC = major histocompatibility complex, APC = antigen presenting cell ; IL=interleukin ; Cyt = cytokines.

Etanercept

Etanercept is a fusion protein produced by recombinant DNA technology through linking the human gene for soluble TNF receptor 2 to the gene for the Fc component of human immunoglobulin G1 (IgG1). Expression of the construct produces a continuous large protein "fusing" TNF receptor 2 to IgG1. It has a molecular weight of 150 kDa. Etanercept behaves like naturally occurring soluble TNF-α receptor and acts by binding to TNFα. This action is similar to naturally occurring interaction between TNF-α and its receptor. However, it has a greatly extended half-life in the bloodstream and, therefore, a more profound and long-lasting biological effect than a naturally occurring soluble TNF receptor.

Etanercept has been found useful in various inflammatory disorders including autoimmune diseases such as ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis and rheumatoid arthritis. It is also considered useful in a variety of other disorders mediated by excess TNFα. Similar to other TNF alpha inhibitors, etanercept use in human patients has been associated with serious and sometimes fatal blood disorders, hepatoxicity, infections, and rare reports of lymphoma and solid tissue cancers. Rare reports of demyelinating central nervous system disorders and congestive heart failure have also been received.

Abatacept

Abatacept is a fusion protein composed of an immunoglobulin fused to the extracellular domain of CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4, also known as CD152). Abatacept is a selective costimulation modulator as it inhibits the costimulation of T cells. It has been approved for the treatment of rheumatoid arthritis in the case of inadequate response to anti-TNFα therapy.

Other fusion proteins

Some other fusion proteins have been developed artificially by recombinant DNA technology. These include aflibercept, alefacept, belatacept, pegsunercept and rilonacept.

2. Androgenic Steroids

Danazol

Danazol is a synthetic derivative of ethinyl testosterone, which possesses androgenic activity. It has a synergistic action with glucocorticoids in the control of immune-mediated thrombocytopenia or haemolytic anaemia (Type II immune complex disease). Although the exact mechanism of immunosuppression is not defined, it is suggested that danazol reduces number of immunoglobulins (Fc) receptors on the surface of macrophages and causes a decrease in the amount of antibody on surface of target cells. The onset of action of danazol may vary from 2 weeks to 2 months; once the patient is stabilised, the dose of glucocorticoid may be gradually reduced and animal may be maintained on danazol. Important adverse effects of danazol in dogs may include hepatotoxicity, increased muscle mass, testicular atrophy and virilisation in females (see for detail Chapter 52).

Dose

For immune mediated thrombocytopenia and haemolytic anaemia

Dogs & cats : 2 – 5 mg/kg, PO, 2 to 3 times daily in combination with prednisolone.

IMMUNOSTIMULANTS

Immunostimulants are drugs which cause increase in magnitude of the immune response. These drugs increase the host resistance to disease affecting cellular or humoral immunity and play an important role in immunodeficiency, chronic infectious disease and cancer. Many immunostimulant drugs are non-specific in their actions and may affect several processes of the immune response. The major indications for pharmacological stimulation of immune system in animals are the prevention or treatment of infections (particularly in immunocompromised host), prevention and therapy of malignancies, hastening maturation of specific and susceptible animals, enhancing level and duration of specific immune responses following vaccination, and overcoming immunosuppressive effects of stress and infectious diseases.

Classification

Immunostimulants are broadly classified into following groups depending on their source or origin.

I. Immunostimulants of microbial origin
   e.g. Bacillus Calmette-Guerin (BCG), muramyl dipeptide, regressin V, Propionibacterium acnes, Staphylococcal protein A and lentinan.

II. Immunostimulants of animal origin
   1. Cytokines
      a. Interleukins
         e.g. interleukin-2 and interleukin-11.
      b. Interferons
         e.g. interferon-alpha, interferon beta1 and interferon-gamma.
      c. Colony stimulating factors
      i. Granulocyte-colony stimulating factor
         e.g. filgrastim and pegfilgrastim.
      ii. Granulocyte-macrophage colony-stimulating factor
         e.g. sargramostim and molgramostim.
      iii. Stem cell factor
         e.g. ancestim.
   2. Hormones
      e.g. thymosin-α and thymopoietin.

III. Immunostimulants of plant origin
   e.g. acemannan.

IV. Synthetic immunostimulants
   e.g. levamisole, cimetidine, isoprinosine and histamine dihydrochloride.

V. Immunostimulants of natural origin
   1. Vitamins
      e.g. vitamin A, vitamin E and vitamin C.
   2. Minerals
      e.g selenium, copper, zinc and cobalt.

VI. Miscellaneous immunostimulants
   e.g. roquinimex, plerixafor, pidotimod, polyinosinic: polycytidylic acid, glatiramer acetate and immunocyanin.

I. IMMUNOSTIMULANTS OF MICROBIAL ORIGIN

A variety of bacterial and fungal products have been used to non-specifically stimulate several aspects of the immune response. Adverse effects can be minimised by administration of killed microorganisms or selected microbial fractions, which stimulate the immune response while avoiding infection.

Bacillus Calmette-Guerin (BCG)

Bacillus Calmette-Guérin (BCG) is a vaccine against tuberculosis that is prepared from a strain of the attenuated (weakened) live bovine tuberculosis bacillus, *Mycobacterium bovis*. It produces generalised enhancement of both B and T cell mediated responses and causes a direct stimulation of the mononuclear phagocytic system. The stimulation of macrophage releases colony stimulating factor and IL-1, which contribute to macrophage regulation and lymphocyte proliferation, respectively. This preparation is active against tumours by unclear mechanisms.

In human medicine, BCG is indicated for treatment and prophylaxis of the carcinoma of urinary bladder, although it can be used for all types of neoplasms. It has been used with some success in equine sarcoids and ocular squamous cell carcinoma. Bacillus Calmette-Guerin has also been used as an adjunct in combination with tumour vaccines for dogs and cats. Adverse effects include hypersensitivity reactions, fever, malaise, shock and immune complex disease. It may also induce a glaucomatous reaction at the site of administration.

Muramyl Dipeptide

Muramyl dipeptide (MDP) is a peptidoglycan fractionated from *Mycobacterium* cell wall. It is composed of N-acetylmuramic acid linked by its lactic acid moiety to the N-terminus of an L-alanine D-isoglutamine dipeptide. Similar to BCG, it non-specifically enhances B-cell, T-cell and macrophage activities. MDP is rapidly cleared after parenteral administration hence it is biologically modified to prolong its action by incorporation into liposomes or conjunction with glycoproteins. When liposomes are used to carry and deliver the drug to macrophages, enhanced macrophage activity is developed against malignant cells. These activated macrophages selectively lyse neoplastic cells, but do not affect non-neoplastic cells. The MDP is useful in treatment of metastasis in dogs with osteocarcinoma.

Regressin V

Regressin V is a proprietary name of an emulsion of mycobacterial cell wall fractions which have been modified to reduce their toxic and allergic effects but retain their antitumour activity. It contains muramyl dipeptide and trehalose dimycolate. Regressin V induces IL-1 and tumour necrosis factor-α secretion from the monocytes and macrophages, and enhances production of antibodies.

Regressin V is recommended for the treatment of mixed mammary tumours and mammary adenocarcinoma in dogs and sarcoids in horses. It is administered by intratumoural injection so as to avoid a generalized response. Fever and malaise may occur after regressin V injection; occasionally the untreated sites also undergo regression. Drowsiness and increased metabolic rate leading to decreased appetite for one to two days following a Regressin V injection is also considered a normal response of drug. Animals with a history of hyper-immune disease (vaccine bumps, allergies, etc.) should be closely monitored for adverse effects. In the event of urticaria, lymphadenitis, cellulitis or abscessation, the animal should be treated appropriately.

Propionibacterium acnes

Propionibacterium acnes (formerly *Corynebacterium parvum*) is a relatively slow growing, typically aerotolerant anaerobic gram positive bacterium that is linked to the skin condition acne. A killed suspension containing *P. acnes* is used in veterinary medicine for non-specific immunostimulation. The cell wall of *P. acnes* contains muramyl dipeptide and lipid components which are immunostimulatory. This material has a complex activity as it stimulates many aspects of immune response including phagocytosis by macrophages and neutrophils, cytotoxicity by natural killer cells and T-cells, development of T and B cells, and production of cytokines like interleukins-1, interferons and tumour necrosis factor. It also increases resistance to infection by many pathogenic bacteria and viruses and enhances bacterial clearance from liver, spleen and blood. It may also induce tumour regression.

Propionibacterium acnes preparation is indicated for the treatment of clinical signs associated with viruses and bacteria induced immunosuppression. It is used in the therapy of dogs with pyoderma, cattle with bovine papilloma and horses with respiratory disease and chronic endometritis. It is also indicated in the treatment of symptomatic feline leukaemia and as adjunct to surgery in the treatment of oral melanoma in the dogs. Important side effects observed in horses may incude fever, anorexia and lethargy within 12-24 hours after first doses.

Staphylococcal Protein A

Staphylococcal protein A (SpA) is a cell wall polypeptide of *Staphylococcus aureus*. Like other bacterial products, SpA produces non-specific immunostimulation. It stimulates production of interleukins-1, interleukins 5, tumour necrosis factor and gamma-interferon and initiates T cell and B cell proliferation. Studies have shown that SpA treatment reduces tumour size, decreases level of circulating immune complexes and induces appearance of cytotoxic antibodies directed towards neoplastic cells. This product has been successfully used in the treatment and management of *Staphylococus* associated pyoderma in dogs. Dogs occasionally become sensitised to the SpA.

Lentinan

Lentinan is a polysaccharide isolated from fungus *Lentinus edodes*. It stimulates macrophage function and interferon production. Lentinan has been developed as an intravenously administered anti-cancer agent, which has been shown to affect host defence immune systems.

Other Products

Products from *Bordetella pertusis*, *Brucella abortus*, *Bacilus subtilis* and *Klebsiella pneumonia* have shown immunostimulating activity. Endotoxins from gram-negative bacteria enhance antibody production, if given at about the same time as the antigen. Endotoxins may also enhance immune activity by promoting release of interferons from cells. Crude bacterial mixtures have been used to stimulate immunity to tumours in animals.

II. IMMUNOSTIMULANTS OF ANIMAL ORIGIN

1. Cytokines

Cytokines are a group of polypeptides which regulate cell growth and differentiation, apoptosis, inflammation, immunity and repair. They regulate and coordinate immune responses by intracellular communication. Recombinant DNA technology has been used to produce cytokines, some of which are commercially available and used in the therapeutics. Cytokines are potent biological response modifiers hence their use in therapy should be carefully regulated. Pharmacological doses of cytokines may perturb many cytokine cascades with resultant side effects and toxicity. Dosing may be critical because low doses of some

cytokines (e.g. interferon) appear to be immunostimulating, whereas high doses are immunosuppressive.

A. Interleukins

Interleukin-2

Human recombinant interleukins-2 (rhIL-2, Aldesleukin) is produced by recombinant DNA technology in *E. coli*. This recombinant form differs from the native human IL-2 in that it is not glycosylated, has no amino terminal alanine, and contains a serine substituted for cysteine at amino acid 125. On parenteral administration, it binds to IL-2 receptors on the surface of responsive cells inducing proliferation and differentiation of helper T cells and cytotoxic T cell. It has also been demonstrated to induce B-cell proliferation, stimulate macrophage activity, increase cytotoxicity of natural killer cells and cause induction of interferon-gamma activity. Several studies have demonstrated that rhIL-2 has activity in companion animals similar to the activity recognised in humans. Infusion of rhIL-2 into normal dogs results in lymphocytosis and enhanced *in vitro* lysis of a canine tumour line.

Clinically, IL-2 has been used for treatment of renal cancer in human beings, oral melanoma in dogs and fibrosarcoma in cats. Intramammary infusion of IL-2 may increase mastitis cure rates. Administration of rhIL-2 has been associated with serious cardiovascular toxicity in humans resulting from capillary leak syndrome, which involves loss of vascular tone and leak of plasma proteins and fluid into the extravascular space. Hypotension, reduced organ perfusion and death have also been reported in human patients. In dogs and cat, adverse effects including fever, inappetance, hypotension and non-cardiogenic pulmonary oedema have been described. Canine IL-2 cDNA and feline IL-2 cDNA have been cloned, but are not commercially available.

Interleukin-11

Interleukin-11 (Oprelvekin) is a pleiotropic (having unrelated multiple effects) cytokine that enhances the activity of primitive, erythroid and megakaryocyte progenitor cells. It simulates production of hepatic acute phase proteins and supports growth of intestinal epithelium. Clinically human recombinant interleukin-11 (rhIL-11) has been used to support platelet levels in human cancer patients and in management of Crohn's disease and ulcerative colitis. In dogs, rhIL-11 is reported to increase platelet counts, platelet size, number of megakaryocytes, and marrow and peripheral blood. A serious adverse effects limiting administration of rhIL-11 in humans is plasma volume expansion resulting in cardiac arrhythmia, oedema and fall in haematocrit values. Pneumonitis may be a dose-limiting side effect in dogs.

Other interleukins

Some human recombinant interleukins have been studied in domestic animals. In dogs, rhIL-6 has been shown to increase platelet counts and enhance sensitivity of platelets to activation in response to thrombin and platelet activating factor. Human recombinant IL-1 (rhIL-1) has been shown to be chemotactic for canine neutrophils. Additional investigations, however, are required to determine the clinical efficacy of these human recombinant products in animals.

B. Interferons

Interferon-alpha

Human recombinant interferon-alpha-2b (rhIFN-alpha2) is available and is obtained from *Escherichia coli* by genetic engineering. It is indicated in the treatment of a variety of tumours in human patients including hairy cell leukaemia, chronic myelogenous leukaemia, Kaposi sarcoma, basal cell carcinoma, renal cell carcinoma and genital warts. It is also indicted for infectious diseases and chronic hepatitis B. Both hepatitis B and hepatitis C are treated with IFN-α, often in combination with other antiviral drugs.

The rhIFN-α has been studied in some animals. In cats, the immunomodulatory effects of rhIFN-α appeared to be dose dependent as its low doses are reported to enhance immune responses, whereas high doses suppress these responses. *In vitro* studies have shown that growth of certain canine and feline tumours are inhibited by rhIFN-α. However, sensitivity to the drug varies according to the type of neoplasm with round cell tumours being most sensitive. The parenteral administration of rhIFN-α in combination with zidovudine (AZT) has been found to be useful in treating retrovirus-induced immunosuppression such as that occurs in feline leucopenia. Important adverse effects of rhIFN-α as observed in human beings include fever, chills, hypotension, arrhythmias and CNS depression.

Dose

Horses : 50 – 150 IU, PO, once daily for 5 days.

Interferon beta-1

Recombinant interferon beta-1a and interferon beta-1b are available commercially for use in human medicine. Interferon beta-1a is produced by mammalian cells while interferon beta-1b is produced in modified *E. coli*. Both types of interferon beta-1 are used to treat multiple sclerosis (MS). They have been shown to slow the progression of disability in MS patients and cause a significant reduction in the rate of MS relapses. It is believed that interferon-beta based drugs achieve their beneficial effect on MS progress via their anti-inflammatory properties. Studies have also determined that interferon-beta improves the integrity of the blood-brain barrier (BBB) that generally breaks down in MS patients.

The most commonly reported side effects in human patients are injection site disorders, flu-like symptoms and blood cell abnormalities. More serious side effects include depression, seizures or liver problems. Physiological tolerance and reduced effectiveness can occur due to the development of antibodies to the drugs and side effects may persist even after discontinuation.

Interferon-gamma

Interferon-gamma (IFN-γ) is a human recombinant polypeptide that stimulates phagocytosis and cytotoxicity by inflammatory and immune cells. In human medicine, it is indicated for reducing the frequency and sensitivity of serious infections associated with chronic granulomatous disease. Similar to rhIFN-α, it has shown to decrease growth of some canine and feline tumours *in vitro* studies. Adverse reactions related to rhIFN-γ in human patients include fever, rashes, GI distress, anorexia, weight loss, myalgia and depression.

C. Colony Stimulating Factors

i. Granulocyte-Colony Stimulating Factors

Filgrastim

Filgrastim is a granulocyte colony-stimulating factor (G-CSF) analogue produced by recombinant DNA technology. The gene for human granulocyte colony-stimulating factor is inserted into the genetic material of *Escherichia coli*. Similar to endogenous G-CSF, filgrastim acts by stimulating the proliferation and differentiation of granulocytes. Filgrastim is mainly used to treat neutropenia induced by cytotoxic chemotherapy and bone marrow transplantation. Filgrastim is also used to increase the number of hematopoietic stem cells in the blood before collection by

leukapheresis for use in haematopoietic stem cell transplantation. Common adverse effects associated with filgrastim use include allergic reactions and pain at the injection site.

Pegfilgrastim

Pegfilgrastim is a PEGylated form of the filgrastim. It is prepared by coupling a 20 kDa Polyethylene glycol (PEG) molecule to the N-terminus of the filgrastim protein. It has a human half-life of 15 to 80 hours, much longer than the parent filgrastim (3 - 4 hours). Similar to filgrastim, the pegfilgrastim is clinically used to stimulate the level of neutrophils in conditions like chemotherapy and bone marrow transplantation.

ii. Granulocyte-Macrophage Colony-Stimulating Factors

Sargramostim

Sargramostim is a recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) produced in yeast. Similar to naturally occurring GM-CSF, it stimulates stem cells to produce granulocytes (neutrophils, eosinophils and basophils) and monocytes. It also functions as an immunostimulator. Sargramostim is primarily used for myeloid reconstitution after bone marrow transplantation. It is also used to treat neutropenia induced by chemotherapy during the treatment of acute myeloid leukaemia.

Molgramostim

Molgramostim is another recombinant granulocyte macrophage colony-stimulating factor. Similar to sargramostim, it stimulates granulocytes production and functions as an immunostimulator.

iii. Stem Cell Factor

Ancestim

Ancestim is a recombinant human stem cell factor (SCF), produced in *E. coli* bacteria by insertion of a gene for soluble human stem cell factor. It has a monomeric molecular weight of approximately 18,500 Da and normally exists as a noncovalently associated dimer. It has 166 amino acid sequence protein identical to the natural SCF, except for the addition of a methionine residue at N-terminal and a nonglycosylated nature. Its actions are similar to those of natural SCF causing stimulation of hematopoiesis. Ancestim is supplied as a sterile, white, preservative-free, lyophilised powder for reconstitution and administration as a subcutaneous (SC) injection.

3. Hormones

Thymic hormones

Thymosin-α and thymopoetin are hormones produced by the thymic endocrine tissue. These are involved mainly in the normal maintenance and balance of immunity. They induce maturation of T cell precursors, promote differentiation and proliferation of mature T cells, and maintain active population of circulating precursor lymphocytes. Several synthetic thymic peptides have been prepared by either chemical means or genetic engineering techniques. These proteins are clinically used primarily to restore the immune system in immunoincompetent patients.

III. IMMUNOSTIMULANTS OF PLANT ORIGIN

Acemannan

Acemannan, a polysaccharide derived from *Aloe vera* (Barbadensis miller) plant, is a potent cytokine stimulator with antitumour and antiviral activity. It stimulates the release of interleukins 1 and 6, tumour necrosis factor-alpha, interferon-gamma and nitric oxide from macrophages leading to tumour apoptosis and necrosis. It also causes enhancement of macrophage phagocytosis and cytotoxicity. Other actions include direct antiviral activities leading to inhibition of virus infectivity and replications. Acemannan is recommended for the treatment of feline leukaemia and fibrosarcoma in dogs and cats. Its intratumoural injection induces tumour encapsulation and necrosis which facilitate surgical excision. No significant adverse effect of acemannan has been reported at the recommended dosage.

Dose

Dogs & cats : 2 mg (total), intratumourally, once weekly for 6 weeks.

: 1 mg/kg, IP, once weekly for 6 weeks.

IV. SYNTHETIC IMMUNOSTIMULANTS

Levamisole

Levamisole (l-Tetramisole) is a phenylimidazothizide broad spectrum anthelmintic mainly used in veterinary practice for the treatment of ascariasis and also hookworm infestation. Various studies have indicated that levamisole stimulates recruitment and functions of macrophages and T-cells, and enhances cell mediated toxicity and cytokine production. It also appears to restore the depressed immune functions of B cell. Levamisole does not appear to have any effect in immunocompetent animals.

Indications for levamisole in human medicine include Hodgkin's disease and rheumatoid arthritis and as an adjuvant chemotherapy of colorectal cancer. It reduces incidence of shipping fever in cattle. It is also reported to reduce incidence of bovine mastitis, endometritis and foetal death. However, the use of levamisole for treatment of cancer in animals is not supported. Levamisole causes adverse reactions typical of excessive cholinergic stimulation. The pharmacology of levamisole has been described in Chapter 68.

Dose

For immunorestoration

Dogs & cats : 2.5 – 5 mg/kg, PO, 3 alternate days per week.

Cimetidine

Cimetidine is histamine H_2 receptor blocker that decreases acid secretion from gastric parietal cells (see Chapter 42). It is reported that cimetidine enhances a variety of immunological functions. It exhibits antiproliferative effects on gastric cancer cells and also appears to inhibit the growth of colorectal carcinoma. The role of cimetidine as immunomodulator is inconclusive.

Inosiplex

Inosiplex is an antiviral drug with immunoenhancing properties. Its antiviral activity is largely due to interferon production. It also increases antiviral activity of natural killer cells, T-cell and monocytes and augments cytokine production. As an immunostimulant, inosiplex helps to restore impaired cell-mediated immune response, in addition to that of humoral immune response. Inosiplex is considered useful in the treatment of AIDS in humans, and new castle disease and avian influenza in chickens.

Histamine dihydrochloride

Histamine dihydrochloride has been used for the prevention of relapse in patients diagnosed with acute myeloid leukaemia (AML). It acts by improving the immune-enhancing properties of IL-2. Laboratory studies have shown that histamine dihydrochloride can also induce immune-mediated killing of leukemic cells. The side-effects of histamine dihydrochloride include transient flush and headache. Low-grade fever and inflammation at the site of injection have also been reported due to immune enhancing properties of IL-2.

V. IMMUNOSTIMULANTS OF NATURAL ORIGIN

1. Vitamins

Vitamin A

Vitamin A plays many essential roles in body and is required for maintaining the cellularity of lymphoid organs (See Chapter 54). It stimulates production of some cytokines like IL-1 by macrophages and IL-2 by lymphocytes. Although the role of vitamin A in immunomodulation in animals has not been clearly defined, its deficiency in pigs has been associated with decrease in antibody formation.

Vitamin E

Vitamin E is a fat-soluble vitamin involved in the structural and functional maintenance of many organs and organ systems (see Chapter 54). It influences immune response in animals by enhancing humoral and cell-mediated immunity. The mode of action of vitamin E in enhancing immunity is not clear, but supplementation of vitamin E serves as significant stimulus of immunity in animals. Calves supplemented with vitamin E have shown high antibody titre to bovine herpes virus-1 following booster vaccination. Deficiency of vitamin E results in immunosuppression.

Vitamin C

Vitamin C is required for various physiological processes and it functions as a cofactor in a number of hydroxylation and amidation reactions (Chapter 55). It also modulates immune response by regulating antigen clearance, interferon production and lymphocyte proliferation. Stress and viral infection have been found to deplete vitamin C concentration in leucocytes. Dietary supplementation of vitamin C augments immunity.

2. Minerals

Selenium

Selenium is an essential trace metal. It is a component of glutathione peroxidase enzyme and is an antioxidant. It facilitates lowering of tissue peroxide levels in body by destroying hydrogen peroxidase. It protects neutrophils from oxidative damage. It also improves humoral immunity. Some studies have shown that sheep and pigs maintained on high selenium diet demonstrate high antibody levels.

Other minerals

Some other minerals like zinc, copper and cobalt play role in inflammatory or immune responses in animals. Zinc plays important role in stabilising membrane structure and thus helps in reducing oxidative damage to cells. Zinc deficiency has been associated with lymphopenia and atrophy of thymus and lymphoid tissues. Copper increases microbicidal activity of peripheral leucocytes. Cobalt is considered essential for neutrophils function.

VI. MISCELLANEOUS IMMUNOSTIMULANTS

A large number of immunostimulants belonging to heterogonous classes of drugs are used for various clinical conditions. These include roquinimex (hydroxyquinoline), plerixafor (heterocyclic compound), pidotimod (thiazolidine), polyinosinic:polycytidylic acid (polyribonucleotide), glatiramer acetate (oligopeptide) and immunocyanin (protein).

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