Introduction to Drug Development

Pharmacology is a science that deals with the *origin, nature, chemistry, effects, and uses* of drugs.

The word 'Drug' denotes any medicinal substance that can cure or prevent disease, relieve symptoms, and provide other benefits.

Sources of Drugs

At one time, the only available drugs were substances extracted from plants or, in some cases, animals.

Almost all the drugs in current use have been developed in the laboratory and are manufactured through various chemical processes. However, many of these drugs are synthetic forms of naturally occurring substances – chemical copies similar to the original substance.

Drug Development Process : A review

Not every compound that is tested in lab is marketed. Before a drug is marketed, it has to undergo several stages of development. A company has to screen through many thousand compounds before it could zero in on the development of a promising compound.

For every 1,000 compounds that are identified by a company, only about 30 show promising results. And for every 30 compounds that show promise, only one may hit the market finally.

Drug development is very slow process due to the numerous steps a drug has to go through before it is ultimately launched in the market.

The average time span for a new drug development may include:

- About six-and-a-half years of discovery, preclinical testing, and toxicity studies
- One-and-a- half years in Phase I trials to assess safety in healthy volunteers
- Two years in Phase II trials with a few hundred patients to evaluate the drug's effectiveness and side effects
- Three- and-a-half years in Phase III trials involving thousands of patients and scores of research centers to confirm effectiveness and evaluate long-term effects
- One-and-a- half years of Drug Authority's review of all the clinical trial data

Stages of Development of a New Drug:

Preclinical stage:

This stage involves study on animals to find out various parameters for a drug under development. During preclinical drug development, a company evaluates the drug's pharmacological and toxic effects through *in vitro* and *in vivo* laboratory animal testing.

Pharmacological Evaluation

The *in vivo* animal studies and *in vitro* studies ascertain various parameters, including:

- Effects of the drug i.e. Pharmacodynamics
- Pharmacokinetics i.e. absorption/distribution/metabolism/excretion (ADME); identification of metabolites, bioavailability, principal route of admin and excretion
- Toxicology/Toxicokinetics

The pharmacological actions of a drug are assessed either *in vitro* using isolated cells /organs, or by receptor-binding characteristics as well as *in vivo* studies in animals.

Toxicological Evaluation

Pre-clinical studies also define potential toxicity of a drug by answering questions like:

- What is safe starting dose?
- What is safe stopping dose?
- What organ/systems are at risk?
- Are toxicities monitorable? Reversible?
- Is it potentially carcinogenic?

Toxicity studies are usually carried out in two appropriate species – one rodent, one second species (dog, pig or monkey generally). Use of highest dose is to demonstrate Maximum Tolerated Dose (MTD) and a lower dose for No Adverse Effect Level (NOAEL).

Exposures achieved in test species should be sufficient to cover multiples of the intended human dose/exposure in order to establish a safety profile.

Acute toxicity studies ascertain LD_{50} in rats and mice.

Subacute toxicity studies assess the toxic effects after 6-13 weeks of administration of a drug in rats at 3 drug levels and 4-13 weeks administration in dogs at 3 drug levels.

Chronic toxicity studies involve 1 year administration in rats at 3 drug levels and 6 month administration in dogs at 3 drug levels. Reproductive studies in rats and rabbits are also conducted to evaluate the effects on male/female fertility and teratogenicity.

Carcinogenicity potential assessment is carried out for drugs having special cause for concern or for those that are intended for chronic use.

Clinical Stages:

Phase 0

This phase has been recently designated. The purpose of conducting Phase 0 study is to expedite early phase 1 studies to make drug development process more efficient.

It needs to be made clear that this is a method for choosing most promising drug candidate and there is no therapeutic benefit involved. Also all drug candidates may Not be appropriate for phase 0 testing.

This phase helps reduce the time and resources needed to distinguish between those potential candidates that hold promise and those that do not.

This is done by selecting a candidate with most favourable properties for further clinical testing and eliminating 'bad' candidates early in clinical development due to bad Pharmacodynamics (PD) or Pharmacokinetics (PK), such as lack of proper effect, poor bioavailability, rapid clearance etc.

This phase shows whether mechanism of action defined in pre-clinical models can be observed in humans and provides human PD/PK data before definitive phase 1/ 2 studies are conducted for potential treatment of a disease.

Micro-dosing phase 0 studies use subpharmacological doses to obtain basic pharmacokinetic data such as volume of distribution, clearance, half-life etc. subject to availability of ultrasensitive analytical methods.

Phase I

Phase I studies are carried out in healthy volunteers, which are small in number – usually 20 to 100. The purpose is to identify metabolic and pharmacological effects of drug in humans and to determine the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. The phase I studies mainly determine safety profile.

Phase II

Phase 2 includes the early controlled clinical studies conducted in a relatively small number of patients to obtain some preliminary data on the effectiveness of the drug for a particular indication or indications in patients with the disease condition. This phase of

testing also helps determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well-controlled and closely monitored.

Phase III

Phase 3 studies are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase 2, and are intended to gather additional information about effectiveness and safety needed to assess the risk- benefit ratio of the drug. Phase 3 studies usually include several hundred to several thousand people.

Phase IV

In addition to these three phases, Phase IV, also known as Post Marketing Surveillance is also carried out once the drug is approved and marketed. The aim of Phase IV study is to find out safety profile in large patient pool across the world and to establish the safety profile of the drug.

Introduction to Drugs – Properties, Formulations & Manufacture

Physicochemical properties of a drug influence the entry of a drug into the body and its subsequent actions.

Physical properties of Drugs

The important physical properties of a drug are:

- Solubility and permeability
  Solubility of a drug is the first requirement for drug absorption in the gastro-intestinal (G-I) mucosa. Less or no solubility means little or no absorption in the body and hence, little or no response / effect.

  Permeability regulates how substances travel across a cell membrane. Poor permeability means poor absorption of a drug in G-I tract and poor distribution across body tissues.

- Physical state

  Physical state of a drug affects the rate and extent of its absorption.

  Order of absorption of a drug based on its physical state from fast to slow is as follows – solution $\longrightarrow$ emulsion $\longrightarrow$ suspension $\longrightarrow$ capsules $\longrightarrow$ tablets enteric coated tablets $\longrightarrow$ modified release tablets/capsules

- Polarity

  A drug needs to be in 'polar' (hydrophilic) state to be soluble in water. On the other hand, to get across a cell membrane, a drug should be 'non Polar' (lipophilic).

- Particle size

  Smaller particle size of a drug means higher dissolution in G-I tract and results in faster absorption.

Chemical properties of Drugs

- Partition coefficient

  For a drug to get absorbed it has to dissolve in lipid layer of G-I mucosa. Low lipid solubility leads to poor absorption of the drug.

- Isomerism

  Many drugs are optically active and have different actions based on the spatial arrangement of the molecule.

  Diastereomers - Dextrorotatory isomer Dextorphan is an antitussive providing relief from cough while Laevorotatory isomer Levorphanol is an analgesic.

  Enantiomers - R Naproxen is inactive while S Naproxen is an NSAID.

- Ionization

  Ionized drug has higher water solubility but low lipid solubility while non-ionized drug has low polarity, high lipid solubility and high permeability.

  Ionized form of a drug is more water soluble – a requirement for administration or distribution of drug in the body while in non-ionized form a drug can move across cell membrane.

- pH

  Chemically, most drugs are weak acids or weak bases.

  An acidic drug dissolves in a basic medium and a basic drug dissolves in an acidic medium.

  pH of different body fluids is as follows: plasma - 7.35 to 7.45, stomach – 1 to 3, small intestine - 7.5 to 8

Drug Formulations

Formulation studies consider such factors as particle size, polymorphism, pH, and solubility etc., as all of these can influence bioavailability and hence the activity of a drug.

Most drugs are specially prepared in a form designed for the convenience of administration. This helps to ensure that doses are accurate and that taking the medicine is as easy as possible.

Oral Preparations

Tablet

This contains the drug compressed into a solid dosage form. They are of different shapes. In some tablets, the active ingredient is released slowly after the tablet has been swallowed whole, to produce a prolonged (‘*sustained’*) effect.

Capsule

The drug is contained in a cylinder-shaped gelatin shell that breaks open after the capsule reaches the stomach and the drug is released. Slow- or sustained-release capsules contain small pellets that dissolve in the small intestine and release the drug gradually.

Liquids

Some drugs are available in liquid form. The active ingredient is combined in a solution, suspension, or an emulsion with other substances like solvents, preservatives, and flavouring and/or colouring agents.

Injectable (Parenteral) Preparations

Solutions for injections are sterile (germ-free) preparations of a drug dissolved or suspended in a liquid. The injections are usually given intramuscularly or intravenously. The other less commonly used routes are – subcutaneous (below the skin), intradermal (within skin layers), intrathecal (into the spine) etc.

Local preparations

Topical Skin Preparations

These preparations are designed for application to the skin for producing local effects. These include:

- Cream

  A cream is a non-greasy semisolid preparation used for applying a drug to the skin. It is less noticeable than an ointment.
- Ointment

  An ointment is a greasy semisolid preparation used to apply a drug to the skin surface. It provides protective and lubricating effect for the relief of dry skin conditions.
- Lotion

  A lotion is a solution or suspension applied to unbroken skin to cool or dry the affected area

Eye Drops

A sterile solution or suspension of a drug instilled in the eye to provide relief from eye diseases.

Ear Drops

A sterile solution or suspension of a drug put in the ear to provide relief from ear disorders.

Nasal Drops

A sterile solution or suspension of a drug introduced in the nose to provide local effect.

Miscellaneous Preparations

Suppositories & Pessaries

Suppositories and pessaries are solid, bullet-shaped dosage forms specially designed for easy insertion into the rectum (rectal suppository) or vagina (pessary). The active drug is gradually released in the rectum or vagina, respectively, as the suppository or pessary dissolves at body temperature.

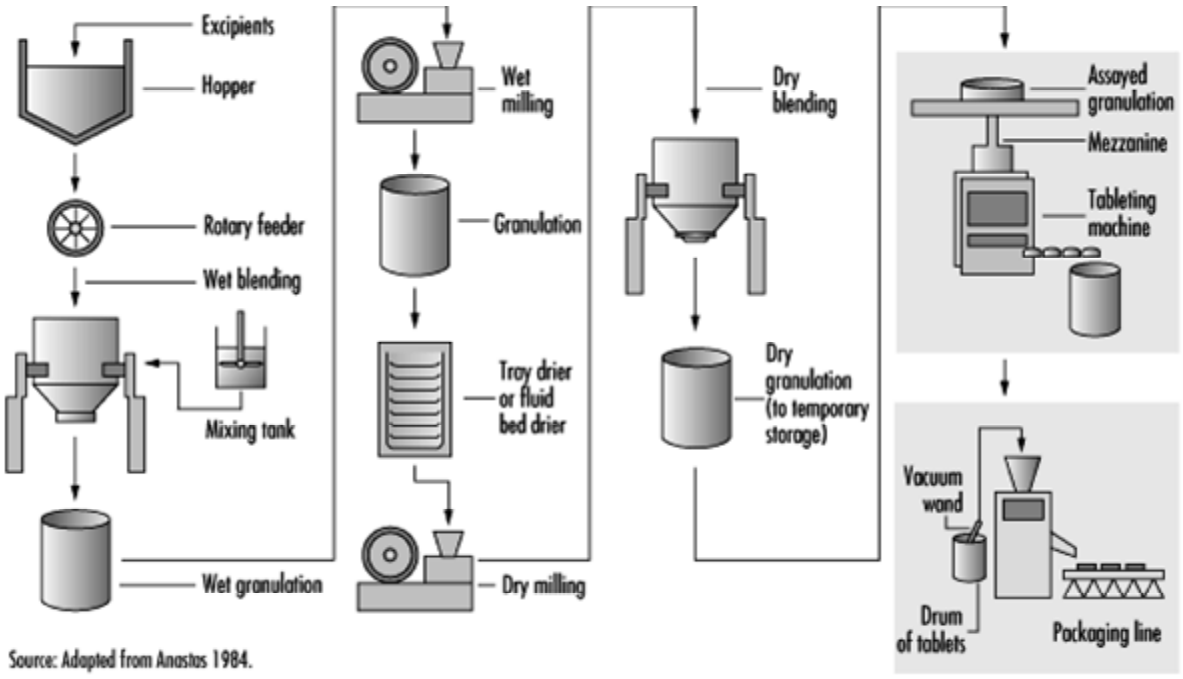
Manufacture of Drugs

Active drug substances and inert materials are combined during pharmaceutical manufacturing process to produce various dosage forms e.g. tablets, capsules, liquids, powders, creams and ointments.

Pharmaceutical ingredients e.g., binders, fillers, flavouring and bulking agents, preservatives and antioxidants, colouring, diluting agents, emulsifiers and suspending agents, ointment bases, pharmaceutical solvents and excipients etc. are mixed with active drug substances, providing the desired physical and pharmacological properties in the dosage form to be manufactured.

These ingredients may be dried, milled, blended, compressed and granulated to achieve the desired properties before they are manufactured as a final formulation.

Tablets and capsules are very common oral dosage forms.



Typical oral tablet manufacturing process flow

Pharmaceutical blends may be compressed by wet granulation, direct compression or slugging to obtain the desired physical properties, before their formulation as a finished drug product.

In wet granulation, the active ingredients and excipients are wetted with aqueous or solvent solutions to produce coarse granules with large particle sizes. The granules are dried, mixed with lubricants (e.g., magnesium stearate), disintegrants or binders and then compressed into tablets.

During direct compression, a metal die holds a measured amount of the drug blend while a punch compresses the tablet.

Drugs that are not sufficiently stable for wet granulation or cannot be directly compressed are slugged. Slugging or dry granulation blends and compresses relatively large tablets which are ground and screened to a desired mesh size, then recompressed into the final tablet.

Some tablets also undergo a coating process, in which layers of edible wax and sometimes sugars are used to seal the tablet.

The tablets are packaged by sealing them between layers of aluminium foil and plastic film (blister-packaging) or they are bottled depending on the nature of the product. The blister packs or bottles are then conveyed along a line on which they are inspected and placed in pouches or boxed with appropriate inserts.

Blended and granulated materials may also be produced in capsule form. Hard gelatin capsules are dried, trimmed, filled and joined on capsule-filling machines.

Liquids may be produced as sterile solutions for injection into the body or administration to the eyes; liquids, suspensions and syrups for oral ingestion; and tinctures for application on the skin.

Highly controlled environmental conditions, contained process equipment and purified raw materials are required for manufacturing sterile liquids to prevent microbiological and particulate contamination. Water at high temperatures and pressures is used to destroy and filter bacteria and other contaminants from the sterile water supply when making solutions for injection.

Parenteral liquids to be injected by intradermal, intramuscular or intravenous administration into the body and solutions to be administered to the eyes (ophthalmic) are sterilized by dry or moist heat under high pressure with bacteria-retaining filters.

Although liquid solutions for oral or topical use do not require sterilization, solutions to be administered to the eyes (ophthalmic) must be sterilized.

Oral liquids are prepared by mixing the active drug substances with a solvent or preservative to inhibit mold and bacterial growth.

Liquid suspensions and emulsions are produced by colloid mills and homogenizers, respectively.

Creams and ointments are prepared by blending or compounding active ingredients with petrolatum, heavy greases or emollients before packaging in metal or plastic tubes.

Pharmacodynamics - Mechanisms of Drug Action

Pharmacodynamics is the study of the biological and therapeutic effects of drugs.

This involves consideration of what drugs do and how they do it, that is, the nature and mechanism of drug actions.

Drug Action

Drugs serve different purposes – sometimes curing a disease and sometimes only relieving symptoms. Their impact often occurs in various parts of the body.

Although different drugs act in a different ways, their actions generally fall in one of the categories given below:

1. Physical activity

Some drugs act by virtue of their physical properties e.g. adsorption of toxins by charcoal or osmotic changes induced by certain laxatives.

2. Chemical activity

Some drugs produce their effect purely due to their chemical properties e.g. neutralization of stomach acid by antacids or combating acidosis by sodium bicarbonate.

3. Enzyme inhibition

Enzymes are substances that regulate the rate of chemical reactions. A drug may stop enzymatic action by competitive or non-competitive inhibition.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like ibuprofen & aspirin act by inhibition of the enzyme Cyclooxygenase (COX). This stops Arachidonic acid conversion to Prostaglandins that mediate the inflammatory response.

Other examples of enzyme inhibition include HMG-CoA Reductase Inhibitors for hypercholesterolemia e.g. atorvastatin, pravastatin and Angiotensin Converting Enzyme (ACE) Inhibitors for high blood pressure, heart failure, and chronic renal insufficiency such as lisinopril, ramipril etc.

Some drugs act as false substrates for an enzyme. The drug molecule is converted by endogenous enzymes to an abnormal substance that enters and disrupts normal metabolic pathways. For example, Fluorouracil replaces uracil so that purine not formed leading to DNA synthesis getting blocked and hence, no cell division.

Some drugs may cause enzyme Induction e.g. increase in metabolism of other drugs by Barbiturates by induction of CYP2B enzyme system in liver.

4. Inhibition of Ion channels

Many drugs inhibit ionic movement across excitable cell membranes e.g. Nifedipine blocks calcium channels and Sodium Channel Blockers that suppress cardiac arrhythmias like lidocaine & amiodarone.

This is achieved by inhibition of enzyme activity usually by drug interaction at a receptor site.

5. Replacing chemical substances that the body lacks

In order to function normally, the body requires sufficient levels of certain chemical substances. These include vitamins and minerals that are obtained from food.

A balanced diet usually supplies what the body needs. However, when the diet does not provide the required amounts of these substances it results in various deficiency disorders. For example, a lack of vitamin C causes a condition called scurvy, lack of vitamin D in children leads to rickets, and iron deficiency results in anaemia.

In case of some hormones produced by the body similar deficiency status may follow when their production is not enough to meet the body requirement. For example, low levels of thyroid hormones leads to hypothyroidism or goiter and a less insulin secretion results in a type of diabetes.

Deficiency disorders are treated with drugs that replace the substances that are missing or in case of hormone deficiencies with their animal or synthetic replacements.

6. Drug-receptor interaction

A majority of drugs act through their binding to a specialized constituent of a cell and altering its functions & functions of the system to which it contributes.

One such specialized constituent of a cell is the *'receptor'*.

Receptors are specialized element of a cell or a tissue with which a drug interacts to produce its characteristic pharmacological actions.

Structurally, receptors appear to be large molecules such as proteins, enzymes, or lipoproteins. The interaction between a drug and a receptor triggers a series of events that alter body systems leading to a pharmacological effect of the drug.

A drug that reacts with a specific receptor may have multiple effects based upon the organ/s in which the receptor is located.

Many natural body chemicals such as hormones and neurotransmitters also interact with receptors producing a response from the cell.

Drugs that bind to receptors and add to the effect of the body's natural chemicals thereby enhancing cellular response are called '*agonists'*.

Drugs that bind to receptors and prevent the body's natural chemicals from binding to the receptors thereby blocking the cell response are called '*antagonists'*.

Competitive antagonist competes with an agonist for the receptor site e.g. agonist Isoproterenol and the antagonist Propranolol bind reversibly to the β-adrenoceptor.

Noncompetitive antagonist binds to a site other than the agonist-binding site (called the allosteric site) of the receptor. $GABA_A$ receptor has a benzodiazepine binding site. Diazepam is an agonist at the benzodiazepine regulatory site, and its antidote flumazenil is an antagonist.

Irreversible Antagonist binds permanently to the receptor binding site by forming a chemical bond that cannot be overcome by an agonist. Phenoxybenzamine binds irreversibly by covalent bonds to α-adrenergic receptors and blocks the activity of norepinephrine.

Physiological antagonism involves drug activation of two different compensatory mechanisms that exist to maintain homeostasis

For example, the effect of norepinephrine to increase blood pressure via stimulation of **α**-adrenergic receptors can be antagonized by administration of acetylcholine, which causes vasodilation by stimulating muscarinic receptors, resulting in the release of nitric oxide from the arteriolar endothelium.

Acetylcholine and norepinephrine exert their effects through different receptors and signal transduction pathways, which produce opposing effects (e.g. vasodilation vs vasoconstriction). They therefore "physiologically" antagonize each other's effects without interacting with the same receptors.

Pharmacokinetic antagonism occurs when one drug accelerates the metabolism or elimination of another. For example, phenobarbital-induced enzyme induction increases the metabolism of the anticoagulant Coumadin.

Partial agonist is a drug which does not produce maximal effect even when all of the receptors are occupied by it.

Buprenorphine is an opioid partial agonist that produces significant analgesic effects by stimulating opioid receptors. Yet, it has a much lower risk for producing life-threatening respiratory depression as compared to a full agonist such as morphine in case of overdose.

Acebutolol is a "beta blocker" that has additional "intrinsic sympathomimetic activity" (ISA). It behaves as a partial agonist at β-1 receptors i.e. ISA results in a neutral effect on heart rate and cardiac output when the sympathetic nervous system is not activated at rest. However, competitive antagonist effect blunts the increase in heart rate when the sympathetic system is activated during times of stress or exercise.

Mixed agonist / antagonist or a selective receptor modulator (SRM) is a type of drug that has different effects in different tissues. A SRM may behave as an agonist in some tissues while as an antagonist in others. For example, Tamoxifen is a widely used SERM in the treatment of breast cancer

Intrinsic activity (IA) or efficacy refers to the relative ability of a drug-receptor complex to produce a maximum functional response. This must be distinguished from the affinity, which is a measure of the ability of the drug to bind to its molecular target, and the EC_{50}, which is a measure of the potency of the drug and which is proportional to both efficacy and affinity.

Potency is the amount of agonist needed to elicit a desired response. The potency of an agonist is inversely related to its EC_{50} value. The EC_{50} for a given agonist is the concentration needed to elicit half of the maximum biological response of the agonist. The EC_{50} value is useful for comparing the potency of drugs with similar efficacies producing physiologically similar effects. The smaller the EC_{50} value, the greater the potency of the agonist and lower the concentration of drug that is required to elicit the maximum biological response.

Drug Target Receptors	Description	Example(s)
Channel-linked receptors	Coupled directly to an ion channel. Activation opens the channel, making a cell membrane permeable to specific ions. These channels are known as ‘ligand-gated’ because it is receptor binding that operates them (in contrast to ‘voltage-gated’ channels that respond to changes in membrane potential) (see B in figure).	Nicotinic acetylcholine receptors; gamma-Aminobutyric acid (GABA) receptors
G-Protein coupled receptors	Coupled to intracellular effector mechanisms via a family of closely related 'G-proteins' that participate in signal transduction by coupling receptor binding to intracellular enzyme activation or the opening of an ion channel. Secondary messenger systems include the enzymes, adenylyl cyclase and guanylyl cyclase, which generate cyclic AMP and cyclic GMP, respectively (see A in figure).	Muscarinic acetylcholine receptors; beta-Adrenoceptors, Dopamine receptors; 5-hydroxytryptamine (Serotonin) receptors; Opioid receptors
Kinase-linked receptors	Linked directly to an intracellular protein kinase that triggers a cascade of phosphorylation reactions.	Insulin receptors
Nuclear hormone receptors	Intracellular and also known as 'nuclear receptors’. Binding of a ligand promotes or inhibits synthesis of new proteins, which may take hours or days to promote a biological effect.	Steroid hormone receptors; Thyroid hormone receptors; Vitamin D receptors

7. Acting on microorganisms invading the body

Infectious diseases are caused by microorganisms that attack the human body. We now have a wide choice of drugs that destroy these microorganisms either by halting their growth or by killing them.

8. Unconventional Mechanisms of Action

- Disrupting of Structural Proteins

  e.g. vinca alkaloids for cancer, colchicine for gout
- Being Enzymes

  e.g. streptokinase for thrombolysis
- Covalently Linking to Macromolecules

  e.g. cyclophosphamide for cancer
- Binding Free Molecules or Atoms

  e.g. drugs for heavy metal poisoning, infliximab (anti-TNF)
- Being Antigens

  e.g. vaccines
- Having Unknown Mechanisms of Action

  e.g. general anesthetics

Placebo Response

The word *placebo* – Latin for *'I will please'* – is used to describe any chemically inert substance given as a substitute for a drug. Any benefit gained by taking a placebo occurs because the person taking it believes that it will produce good results.

New drugs are almost always tested against a placebo preparation in clinical trials as a way to assess the efficacy of a drug before it is marketed. The placebo is made to look identical to the active drug preparation and volunteers / patients are not told whether they have been given the active drug or the placebo. Sometimes, even the doctor is unaware of the preparation that has been given to the individual. This is

known as a '*double blind'* study. In this way a purely placebo effect can be ruled out and the effectiveness of the drug preparation determined more objectively.

Sometimes the mere taking of a medicine has a psychological effect that produces a beneficial physical response. This type of placebo response can make an important contribution to the overall effectiveness of a chemically active drug.

Tolerance

Tolerance is said to have developed when it becomes necessary to increase the dose of a drug to obtain an effect that was achieved earlier with a smaller dose.

Tolerance can be natural or 'acquired'. Natural tolerance is not induced by the drug but is seen due to inherent genetic factors in the individual. Acquired tolerance, on the other hand, occurs after the drug has been given for some time. It is due to changes occurring at the receptor site or due to increased metabolic inactivation of the drug resulting from enzyme induction leading to lower blood levels of active drug.

Intolerance, on the other hand, means a low threshold of response to the pharmacological actions of a drug.

Individuals vary greatly in their susceptibility to drugs – those at one extreme being intolerant of the drugs while those at the other end exhibit tolerance.

Summation and Synergism

When two drugs with similar mechanisms are given together, they typically produce additive effects. This is also referred to as summation.

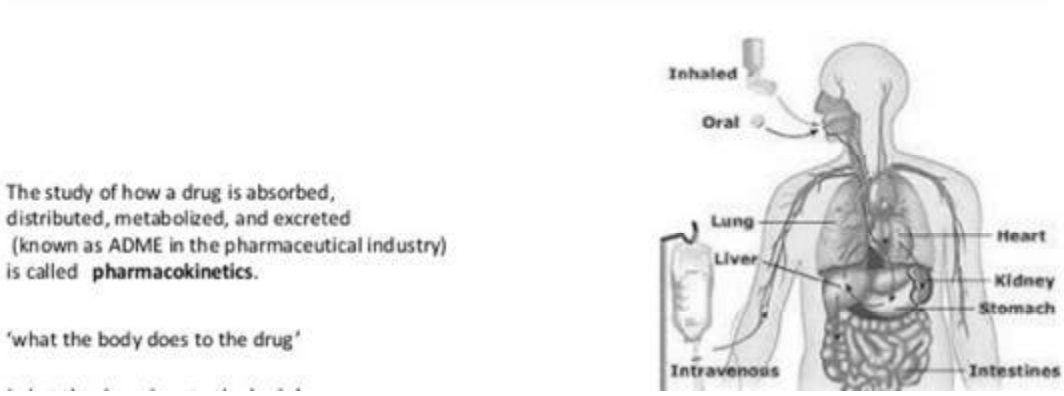
However, if the effect of two drugs exceeds the sum of their individual effects, this is referred to as synergism (or 'Potentiation').

Pharmacokinetics

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of a drug.

As we have seen earlier, Pharmacodynamics deals with the study of biological and therapeutic effects of a drug.

The difference between these two can be put simply as follows: Pharmacodynamics is what the *drugs do to the body* whereas Pharmacokinetics is what the *body does to the drugs*.



Absorption of a Drug

Absorption of a drug is defined as the process of drug movement from the site of administration towards the systemic blood circulation.

The way in which a drug is absorbed depends on its route of administration.

Routes of Drug Administration

The majority of drugs must be absorbed into the bloodstream in order to reach the site where their effects are needed.

A drug is usually administered in one of the following ways – through the mouth or rectum, by injection, or inhalation. Drugs implanted under the skin or enclosed in a skin patch also enter the bloodstream.

When it is unnecessary or undesirable for a drug to enter the blood circulation in large amounts it may be applied topically so that its effect is limited mainly to the site of the disorder e.g. surface of the skin or mucous lining of the nose, eyes, ears, vagina or rectum.

The different routes of drug administration are as follows:

a) Administration by mouth (Oral Route)

Giving drugs by mouth is the most frequently used method of administration. Most drugs that are given by mouth disintegrate and dissolve in the digestive tract and are then absorbed into the bloodstream through the walls of the intestine.

Some drugs (like antacids which neutralize stomach acidity) are taken by mouth to produce a direct effect on the stomach or digestive tract.

b) Sublingual Administration

A tablet placed below the tongue (‘*sublingual*’) on the floor of the mouth results in the rapid absorption of the drug into the bloodstream as the lining of the mouth has a rich supply of blood vessels.

c) Rectal Administration

Drugs intended to have a systemic effect may be given in the form of suppositories inserted into the rectum from where they are absorbed into the blood circulation.

d) Inhalation

Drugs may be inhaled to produce a systemic effect or a local effect on the respiratory tract.

Drugs administered by nasal route include calcitonin (for osteoporosis), sumatriptan (for migraine headaches).

Drugs administered by inhalation through the mouth may act specifically on the lungs, such as antiasthmatic drugs like salbutamol.

Gases to produce general anaesthesia are administered by inhalation and are absorbed into the bloodstream through the lungs to produce a general effect on the body, particularly the brain.

e) Administration by Injection

Drugs may be injected into the body to produce a systemic effect. One reason for injecting drugs is the rapid response that follows.

The main types of injection are intramuscular, intravenous, and subcutaneous.

f) Topical Application

For treating localized disorders such as skin infections and eye / ear infections it is preferable to use drugs in a suitable dosage form so that the drug has a local ('*topical*') rather than a systemic effect.

For example, artificial tears are used to relieve dry eyes, betaxolol eye drops used to treat glaucoma, and drugs used to dilate pupils, such as phenylephrine and tropicamide produce a local effect after they are absorbed through the cornea and conjunctiva.

Ear drops containing solutions or suspensions are typically applied to the outer ear and little of the drugs enter the bloodstream; drugs given by this route include hydrocortisone, ciprofloxacin, and benzocaine.

Cutaneous application

Drugs applied to the skin are usually used for their local effects. Most commonly used drugs to treat superficial skin disorders include hydrocortisone & betamethasone for psoriasis; hydrocortisone, dexamethasone for eczema; antiviral e.g. acyclovir, antibacterial e.g. mupirocin, and antifungal e.g. clotrimazole for skin infections; and urea & liquid paraffin for Itching and dry skin.

Vaginal route

Some drugs may be administered vaginally to women as pessaries (vaginal tablets) e.g. clotrimazole in the topical treatment of vaginal candidiasis or to give estrogen to women after menopause to relieve vaginal symptoms such as dryness, soreness, and redness.

It is much easier to control the effects of drugs administered locally and to ensure that they deliver maximum benefits with minimum ill effects.

Factors that affect the oral absorption of a drug:

- Presence of food or other drugs in the G-I tract

  Presence of food in the G-I tract delays absorption of Aspirin, paracetamol, diclofenac while it decreases absorption of oral penicillins, erythromycin, and tetracyclines. However, food Increases absorption of griseofulvin & diazepam.

- Time taken for passing of stomach contents into the small intestine ('*gastric emptying time*')
  Food, especially fatty food, slows gastric emptying and rate of drug absorption. On the other hand, taking some drugs on an empty stomach speeds up absorption.

Drugs that affect gastric emptying e.g. parasympatholytic drugs like atropine affect the absorption rate of other drugs.

- Time duration for which the drug remains in the intestines (residence time) also affects absorption. Prolonged residence time may increase absorption of Vitamins.

- pH of the G-I tract

  Acidic pH of stomach degrades Penicillin G and erythromycin and hence, they are administered as prodrugs namely carindacillin and erythromycin estolate.

  Acidic drugs (Aspirin) are better absorbed in stomach (in acidic pH) and basic drugs (Diazepam) are better absorbed in intestine (in alkaline pH).

- Diseases of the G-I tract

  For example, Achlorhydria may lead to inhibition of absorption of Vit B_{12}.

Distribution of a Drug

After a drug enters the general circulation it gets distributed throughout the body and passes into various tissues. This is known as distribution of the drug in the body.

Protein Binding

Drugs are transported in the blood partly in solution (as free drug) and partly bound to plasma proteins – mainly albumin. The extent to which a drug binds with albumin depends upon the molecular structure of the drug. Acidic drugs are generally bound more extensively than basic drugs.

Plasma protein binding influences the distribution and pharmacological activity of drugs.

Highly protein-bound drugs include Warfarin- 99% bound, Tolbutamide- 98% bound, and Phenytoin- 90% bound

Free, unbound drug is active and gets metabolized & eliminated. Bound drug dissociates to replace the drug lost from the body.

Displacement interactions where drug bound with higher affinity will displace the one having lower affinity. For example, Phenylbutazone, Salicylates & Sulfonamides displace Tolbutamide resulting in hypoglycemia. Similarly, Salicylates, Indomethacin, Phenytoin & Tolbutamide displace Warfarin increasing the risk of haemorrhage.

Drug Reservoirs

A drug can accumulate in various tissues and serve as drug depots. As the plasma level declines stored drug is released into the circulation prolonging the drug action.

Factors affecting Distribution of Drug

The extent of distribution of a drug in the body depends on many factors, such as:

- Lipid solubility of the drug e.g. Highly lipid- soluble drugs like thiopentone selectively accumulate in fat and adipose tissue
- Variations in the pH levels of body tissues i.e. the pH of the blood or tissue affect the ionization of the drug and hence its distribution e.g. 2nd generation antihistamines are ionized molecules at physiological pH that cross the blood-brain barrier poorly compared to first generation antihistamines (uncharged at pH 7.4)
- Protein binding e.g. extensively protein bound drug like warfarin has smaller apparent volume of distribution.
- Permeability of blood vessels e.g. permeability is increased in renal capillaries and in specialized hepatic capillaries (*sinusoids)* resulting in more extensive distribution to these organs.
- Blood-brain barrier

Capillaries of the brain differ from those in other parts of the body and lack pores present in other capillaries. Also, the connective tissue cells covering around the capillaries (astrocytic sheath). This effectively prevents the passage of drugs and other substances from the blood into the CNS.

Thiopental is only partly ionized and passes into the brain easily.

Metabolism (Biotransformation) of a Drug

Metabolism or biotransformation is the process of chemical alteration of drugs in the body. Metabolism facilitates elimination of the drug from the body. Most of the drugs are eliminated from the body by the kidneys through the urine.

The process of metabolism changes drugs in two major ways:

i. By reducing Lipid Solubility

Metabolic reactions tend to make a drug molecule progressively more water soluble and less lipid soluble. This favours their easier elimination in the urine.

ii. Alteration of Biological Activity

Most drugs are converted by metabolism from a pharmacologically active to an inactive substance or to another pharmacologically active substance. (Sometimes a pharmacologically inactive drug ('*prodrug*') is converted into a pharmacologically active form and exerts its therapeutic benefits).

A number of tissues, such as, kidneys, the lining of digestive tract, lungs, and skin metabolize drugs but the liver is by far the most important organ involved in the metabolism of drugs. The liver cells contain a number of enzymes that are responsible for many metabolic reactions.

Excretion of a Drug

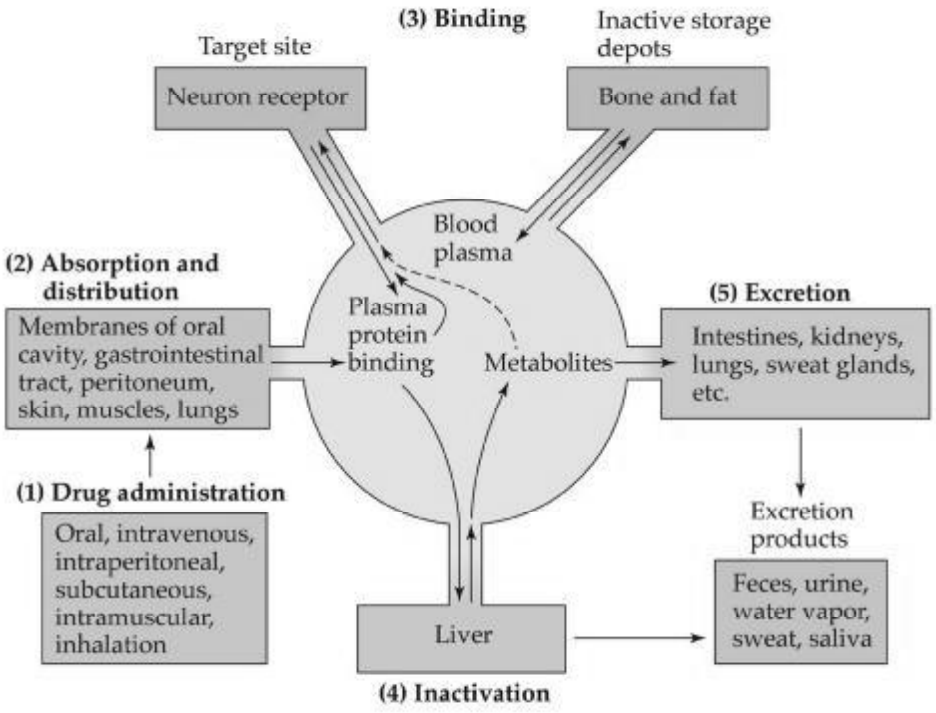
Excretion is the process by which a drug is eliminated from the body.

The major organ responsible for excretion of a drug is the kidney, which eliminates drugs via urine.

Other routes by which drugs are excreted from the body include:

- Bile
- Saliva
- Sweat
- Breast milk
- Lungs, etc.

The following figure explains these events and their interrelationship in a nutshell-



At this stage we need to understand a very important concept in pharmacology, namely, Bioavailability of a drug.

Bioavailability

Bioavailability is the rate and extent to which the drug enters the general circulation. It includes consideration of both amount and rate of absorption into the systemic circulation following the extravascular administration of the drug, for example, administration by oral route.

Bioavailability is determined either by measuring the concentration of the drug in body fluids (viz. blood) or therapeutic response.

Factors affecting Bioavailability of a Drug

Bioavailability of a drug depends on a number of factors, such as –

Pharmaceutical factors

These factors include the way in which a drug formulation is designed and manufactured.

Physicochemical properties of a drug

The solubility of a drug in the gastro-intestinal tract fluids determines its bioavailability as only drugs in solution can be absorbed by the cells of the G-I tract.

Factors related to the patient

These consist of the following:

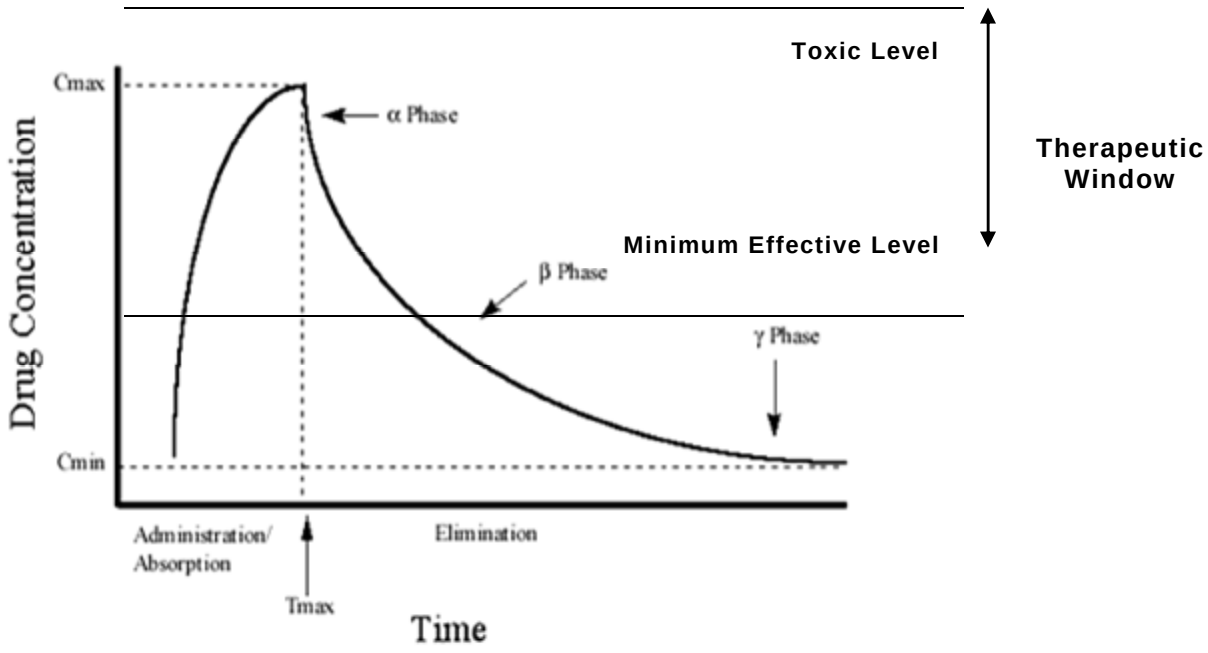
- Presence of food or other drugs in the G-I tract
- Time taken for passing of stomach contents into the small intestine ('*gastric emptying time*')
- Time duration for which the drug remains in the intestines
- pH of the G-I tract
- Diseases of the G-I tract

Estimation of Bioavailability

The extent of bioavailability of a drug is described by using the following parameters:

1) Peak plasma level (*C Max*) – This means the highest concentration of drug achieved in the blood circulation.
2) Time to achieve peal plasma level (*T Max*) – This is the time taken to achieve the highest concentration of a drug in the blood.
3) Area under curve (*AUC*) – this represents the total amount of a drug reaching systemic circulation following administration.

The diagram given below will give a further clarity about these concepts. It is called a 'Time V/s Concentration curve'.



Time V/s Concentration curve

With the help of this diagram we can understand the following important concepts:

Minimum Effective Level –

This is the threshold to be crossed by the drug level in the blood in order to produce its desired effect.

Toxic Level –

This is the upper limit beyond which the drug starts producing harmful effects that may be dangerous.

Therapeutic Window –

This term indicates the range of drug concentration in the blood within which the drug produces its desired effects without causing any harm to the individual.

Half – Life ($t_{½}$) –

Half – life ($t_{½}$) is the time taken for the blood concentration (or the amount of drug in the body) to be reduced by 50 % of the previous reading.

In other words, a constant fraction or percent of drug is eliminated from the body during each unit of time and most of the drug is eliminated from the body after four or five half-lives.

Importance of half-life ($t_{½}$)

A knowledge of half-life is required for :

- Ø Estimation of time required to eliminate a drug from the body after its administration is stopped
- Ø For deciding the dosage schedule
- Ø For prediction of the time required o achieve steady state plasma concentration.

Steady State:

This term denotes a situation when the amount of drug entering the circulation equals that being removed from it. In other words, when the blood concentration of a drug remains more or less same over a period of time a steady state is reached. This is seen after many doses of drug given at fixed intervals.

A steady state is achieved after approximately four to five half-lives.

Toxicology

Introduction

Toxicology is the study of the adverse effects of chemical, physical, or biological agents on people, animals, and the environment.

It is necessary to prove that a new drug is safe before its first administration to humans.

In vitro toxicology studies provide an early indication of the potential for some kinds of toxic effects, allowing a decision to terminate a development program before spending too much money.

In vitro methods are widely used for screening of chemicals and studying cell-, tissue-, or target-specific effects. Although less expensive they are somewhat less predictive of toxicity in intact organisms.

In *in vitro* Cytotoxicity studies, toxicity to cells is assessed and many different types of cells can be used, including cells from higher organisms e.g. liver cells, blood cells etc.

Dermal or ocular toxicity studies, such as Dermal Corrosion, Skin Irritation, and Draize Eye Irritancy can replace *in vivo* tests.

In vivo toxicology methods are used for the following purpose:

- Establish a safe starting dose for clinical studies
- Provide a drug-treatment regimen that would produce the least toxicity
- Assess target organ toxicity and its reversibility
- Provide insight into biomarkers for clinical monitoring

In general, animal studies are conducted in two species, one rodent (e.g., rat, mouse) and one non-rodent (e.g. dog, nonhuman primate).

A drug effect that is seen both in the rat and in the dog probably involves a common physiology mechanism that is likely to be present in the human, whereas an effect seen only in one of the two species indicates that the same is peculiar to that species, and is less likely to be present in the third species.

Other species e.g. rabbits, hamsters, mini-pigs may be used for special studies, such as vaccine studies.

Types of Toxicity studies

Safety pharmacology studies are used to determine the effects of the drug on specialized organ systems (e.g., cardiovascular, respiratory, neurologic)

Acute toxicity studies describe the adverse effects of a drug that may result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours).

Sub-acute toxicity studies show the ability of a toxic substance to cause effects for more than one year but less than the life time of exposed organism.

Chronic Toxicity/Carcinogenicity are used to determine the effects of long-term exposure to the drug, including the ability to produce cancer.

They may not be required for drugs that are intended for only short-term use (e.g., antibiotics) and that are expected to have no permanent effects on DNA.

Reproductive Toxicity/Teratogenicity studies evaluate the effects of a drug on reproductive function and ability to produce birth defects.

Mutagenicity tests evaluate the likelihood of induction of alterations in the information content (DNA) of an organism or cell that are not due to the normal process of recombination at the time of cell division.

Questions that these studies answer

- What are the toxic doses in animals?
- What are the target organs?
- How do the toxic doses compare to the effective/clinical dose(s)?
- Can the toxicities be monitored in patients in the clinical trials?
- Are the toxicities reversible?

Animal Model Requirements in Toxicity Studies

Study Type	Minimum No. of Animals Required		Dosing	Age at Start of Study
	Males	Females		
Acute oral (rat), dermal, or inhalation (rat)	5	5	Single	Young adult
Eye and skin irritation (rabbit)	6[a]		Single	Young adult
Dermal sensitization (guinea pig)	[b]		Repeated	Young adult
21-Day dermal (rat, rabbit, or guinea pig)	5	5	Repeated	Rat, 200–300 g; rabbit, 2.0–3.0 kg; guinea pig, 350–450 g
90-Day oral (rat)	10	10	Repeated	6–8 weeks
90-Day inhalation (rat)	10	10	Repeated	Young adult
90-Day dermal (rat, rabbit, or guinea pig)	10	10	Repeated	Rat, 200–300 g; rabbit, 2.0–3.0 kg; guinea pig, 350–450 g
90-Day or chronic (1 year) oral (dog)	4	4	Repeated	4–6 months
Reproduction (rat)[c]	20	20	Repeated	8 weeks
Teratology				
Rat		20[d]	Repeated	Young adult
Rabbit		12[d]	Repeated	Young adult
Chronic toxicity (1 or 2 year) (rat)	20	20	Repeated	6–8 weeks
Oncogenicity (lifetime) (rat and mouse)	50[e]	50[e]	Repeated	6–8 weeks

[a] Either males or females may be used in this test.

[b] The number of animals used depends on the method used. Several different experimental methods are acceptable.

[c] EPA prefers that one male rat be housed with one female during mating.

[d] Number of pregnant females required.

[e] 50 rats and 50 mice of each sex

ACUTE TOXICITY STUDIES:

An initial step in the assessment of the toxic characteristics of a drug is to use a single dose in each animal only for the determination of gross behavior and LD_{50} or median lethal dose.

LD_{50} value depends on the route of administration. In increasing order - intravenous, intraperitoneal, subcutaneous and oral.

It also helps determine the therapeutic index (LD_{50} /ED_{50}), greater the index, safer is the compound.

The other values that can be assessed are:

- no-observed-adverse-effect level, NOAEL
- Lowest-observed-adverse-effect level, LOAEL
- Maximum tolerable concentration, MTC, Maximum tolerable dose, MTD
- Minimum lethal concentration, LC_{min}; Minimum lethal dose, LD_{min}

Routes of administration of a drug in subacute toxicity studies are oral, dermal, inhalation. Oral and inhalation subacute studies are generally carried out for three months in shorter lived animals (rodents) and 1 year in longer lived animals. Dermal studies are usually performed for 1 month or less.

Subacute Toxicity Studies:

These are designed to examine the adverse effects resulting from repeated exposure over a portion of average lifespan of an experimental animal. A compound found to be non-toxic in Acute toxicity study may be toxic after prolonged exposure at low doses due to accumulation, changes in enzyme levels, and disruption of physiologic and biochemical homeostasis.

Chronic Toxicity Studies:

These assess the ability of a drug to cause harmful effects over an extended period, usually on repeated and continuous exposure. The result of chronic toxicity study in animals should suggest signs and symptoms of adverse reactions to look for in man.

Special Toxicity Studies

These include Teratogenicity and Mutagenicity studies.

Teratogenicity tests

These tests for effects on reproduction involve the study in animals, which have been exposed to the test drug from the time of conception to the time they produce their own offspring plus a study of the offspring during growth and development.

Mutagenicity tests

Mutagenesis is the induction of alterations in the information content (DNA) of an organism or cell that is not due to the normal process of recombination. This change may occur in germ cells or somatic cells.

Somatic mutations in a developing organism may lead to abnormal differentiation of its cells. Alterations in the duplicating somatic cells of an adult may lead to Cancer.

Types of Mutation:

- Point mutations- Alteration in a single nucleotide pair in the DNA molecule viz. A-T or G-C
- Chromosome aberrations- breaks and rearrangement of chromosomes

Mutagenicity studies can be carried out *in vitro* using bacteria e.g. Salmonella typhimurium for mutations at G-C pairs or Escherichia coli for mutations at A-T pairs.

However, a disadvantage is that they lack the physiology and metabolism of mammals. This can be overcome partly by addition of the mammalian metabolizing system (as liver homogenates) to the growth medium.

Animal studies using rats or mice can indicate that genetic damage has occurred in the form of structural or numerical chromosome aberrations. A disadvantage of these tests is that they cannot detect point mutations.

Host Mediated Assay helps detect substances which are not mutagenic *in vitro* but are converted to active mutagens in mammals OR substances may be mutagenic *In-vitro* but get detoxified by mammalian system.

Salmonella are injected intraperitoneally into rat or a hamster. The animal is treated with the test drug orally. Afterwards sample is withdrawn from peritoneal cavity and mutation in salmonella is measured.

The advantage of these tests is that they provide information on the metabolism of mutagens and detect point mutation in mammalian system.

Drug Adverse events/reactions

Sometimes a drug may have effects that do not contribute to any therapeutic benefit and are undesirable, and / or have a potential to cause harm to the patient.

Such 'unwanted' effects of drugs are of three types:

1. Side Effects

Side effects of a drug are the known and frequently experienced, expected reactions to a drug seen at therapeutic doses. Often, these effects are related to the pharmacological actions of a drug. Moreover, a drug that may have been given by mouth, by injection, or by inhalation is distributed throughout the body and hence its effects are unlikely to be restricted to one particular type of tissue, organ or a system.

For example, anticholinergic drugs that are given to relieve painful spasm of bowels may also affect the eye function causing blurred vision, the mouth leading to dryness, and urinary bladder causing retention of urine.

Such side effects may gradually disappear as the body gets accustomed to the drug. But if the side effects persist or become troublesome, the dose of the drug may have to be reduced or dose interval may need to be increased.

2. Adverse Reactions

Adverse reactions are less common unexpected, unpredictable effects of a drug that are not related to the usual pharmacological actions of the drug given at normal therapeutic doses.

Unpredictable drug reactions may be caused by conditions in the patient such as an allergy or a genetic disorder e.g. absence of an enzyme that inactivates the drug in the body.

Common adverse reactions of this type include a rash, swelling of face, or jaundice. They may also be due to interactions with other drugs.

Severe unpredictable drug reactions usually require withdrawal of the offending drug and appropriate treatment.

3. Toxicity

When the blood levels of a drug exceed the toxic level there are harmful effects of the drug that may be dangerous. This may happen due to overdose of a drug or when a person cannot metabolize and / or excrete the drug properly.

Causes of Adverse Drug Reactions

Most adverse drug reactions are dose-related while others are allergic or idiosyncratic.

Dose-related ADRs are usually predictable; ADRs unrelated to dose are usually unpredictable.

Dose-related ADRs are particularly a concern when drugs have a narrow therapeutic index (eg, hemorrhage with oral anticoagulants).

Such ADRs may also result from decreased drug clearance in patients with impaired renal or hepatic function.

Drug-drug interactions may result in ADRs. For example, competition for drug binding site of plasma proteins between warfarin and aspirin may lead to displacement of one drug raising its risk of producing ADRs. Similarly, CYP450 enzymes in the liver can be induced or inhibited by many drugs resulting in altered metabolism of other drugs thereby increasing the risk of ADRs. For example, cimetidine inhibits CYP1A2 enzyme raising the risk of ADRs from administration of propranolol or tricyclic antidepressants.

Allergic ADRs are not dose-related and require prior exposure. Allergies develop when a drug acts as an antigen or allergen. After a patient is sensitized, subsequent exposure to the drug

produces one of several different types of allergic reaction. Clinical history and appropriate skin tests can sometimes help predict allergic ADRs.

Idiosyncratic ADRs are unexpected ADRs that are not dose-related or allergic. They occur in a small percentage of patients given a drug. Idiosyncrasy is an imprecise term that has been defined as a genetically determined abnormal response to a drug.

Classification of Adverse Drug Reactions

Severity	Description	Example
Mild	No antidote or treatment is required; hospitalization is not prolonged.	Antihistamines (some): Drowsiness Opioids: Constipation
Moderate	A change in treatment (eg, modified dosage, addition of a drug), but not necessarily discontinuation of the drug, is required; hospitalization may be prolonged, or specific treatment may be required.	Hormonal contraceptives: Venous thrombosis NSAIDs: Hypertension and edema
Severe	An ADR is potentially life threatening and requires discontinuation of the drug and specific treatment of the ADR.	ACE inhibitors: Angioedema
Lethal	An ADR directly or indirectly contributes to a patient's death.	Acetaminophen overdosage: Liver failure Anticoagulants: Hemorrhage

Drug Treatment in Special Risk Groups

Certain groups of people are at a greater risk when they take drugs. The reason for the same is that the body handles drugs differently or the drug has a typical effect in these individuals.

Patients at special risk are:

- Infants and children
- Pregnant women
- Women who are breastfeeding their babies
- Elderly patients
- Patients with liver or kidney diseases

Infants and children

Infants and children need a lower dosage of a drug than adults because of their lower body weight and differences in body composition.

Pregnant women

Greater care is needed during pregnancy to protect the unborn baby from any harm so that it develops into a healthy newborn.

Drugs taken by the mother can enter the baby's blood circulation. With certain drugs and / or at particular stages of pregnancy, there is a risk of developmental abnormalities, retarded growth or post – delivery problems affecting the child.

Women who are breastfeeding their babies

Just as drugs may cross from the mother's bloodstream to the baby they may also pass through the breast milk. This means that a breastfed baby may receive small amounts of whatever drugs the mother is taking.

In many cases, this is not a serious problem because the amount of drug is too small to have any significant effect on the baby. However, some drugs can produce unwanted effects on the baby. For example, sedative drugs may make the baby drowsy and cause feeding problems.

Elderly patients

Older people are particularly at risk when they take medicines. This is partly due to the physical changes associated with ageing and partly due to the need in some elderly patients to take several drugs at the same time for a multitude of ailments.

They may also be at risk due to their inability to manage their medication on their own or a lack of relevant information.

Elderly people may have a greater risk of accumulating drugs in their bodies because the liver is less efficient at metabolizing the drugs and the kidneys are less efficient at excreting them. As a result, the normal adult doses may produce adverse reactions in some cases and half the adult dose may be sufficient to produce a desired therapeutic effect.

Patients with liver or kidney diseases

Long-term illnesses affect the way in which people respond to drug therapy. This is especially true of kidney and liver disorders.

The liver transforms the complex drug molecules into simpler substances easily removable from the body while the kidneys perform this task of excretion of these substances in urine. The clinical effects of a drug on an individual can be significantly altered if the effectiveness of liver or kidneys is affected by diseases.

People with poor kidney function are at greater risk of adverse reactions to a drug. Firstly, there is a drug accumulation in the body because of smaller amounts of drug are being excreted from the body in urine. Secondly, kidney diseases can cause protein loss in the urine, which lowers the plasma protein binding of drugs rendering a higher proportion of drug free and active in the blood circulation.

Severe liver disease affects the way the body breaks down the drugs. This can again lead to accumulation of drugs in the body. Many drugs may have to be avoided completely since they can cause serious harm to a patient with poor liver function.