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Introduction to Biochemical Pharmacology and Drug Discovery

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<http://dx.doi.org/10.5772/52014>

1. Introduction

This chapter introduces biochemical pharmacology and highlights drug absorption and drug transformation reactions and a general introduction to pharmacology, drug discovery and clinical trials for new drug candidates. It also introduces the concept of individualization of drug therapies. After studying this chapter, one is expected to demonstrate understanding the following: (i) Linkage between the various pharmacological processes (ii) Routes of drug administration, (iii) Mechanisms of drug absorption (iv) The kinetics of drug disposition and concepts, such as volume of distribution, initial dose and half-life, (v) The biotransformation and excretion of drugs. (vi) The role of biochemical knowledge in the discovery and development of candidate drug compounds into useful drugs (vii) Basic design of clinical trials of new drugs and the drug approval process. (viii) The linkage between genetic variations and varied drug responses in different individuals (ix) The various adverse drug reactions in different patients (x) How different dosage regimens are calculated with respect to the prevailing health status of individuals and how adjustments are carried out in old patients or geriatrics.

2. Pharmacology

Pharmacology is the science that deals with drugs, their properties, actions and fate in the body. It embraces the sciences of pharmaceutics (preparation of drugs), therapeutics (treatment of diseases by use of drugs) and toxicosis or adverse side-effects that arise from the therapeutic interventions. Pharmacology can be divided into the following processes:-

- i. The pharmaceutical process of drugs; deals with chemical synthesis, formulation and distribution of drugs.

- ii. Pharmacokinetic process; deals with the time course of drug concentration in the body. This process can be further subdivided into; absorption, distribution, biotransformation and excretion of the drug.
- iii. The pharmacodynamic process; deals with the mechanism of drug action: that is interaction of drugs with the molecular structures in the body.
- iv. The therapeutic process; deals with the clinical response arising from the pharmacodynamic process.
- v. Toxicologic process; deals with adverse effects of drugs arising from either over dosage or interference of biochemical pathways unrelated to the intended drug target. The five processes are related as exemplified in Figure1.

3. Biochemical pharmacology

Biochemical pharmacology is concerned with the effects of drugs on biochemical pathways underlying the pharmacokinetic and pharmacodynamic processes and the subsequent therapeutic and the toxicological processes. The pharmaceutical process is, however, outside the realms of biochemical pharmacology.

4. Routes of drug administration and systemic availability

This depends on the actual biochemical characteristics of the drug and the interaction of drug molecules with body fluids and tissues. The main routes of drug administration are the topical application, parenteral, and enteral routes.

The route of drug application determines how quickly the drug reaches its site of action. The choice of the route of administration of a drug, therefore, depends on the therapeutic objectives of the treatment. For instance, intravenous injection or inhalation may be selected to produce intense, but rather short-lived effects, whereas oral dosing may be better and more convenient for long lasting effects and even intensity. The various types of drug administration include;

4.1. Topical application

This is the most direct and easiest mode of drug administration. It involves local application of a drug to the site of action e.g. eye drop solutions, sprays and lotions for oral, rectal, vaginal and urethral use. These drugs are absorbed through the cell membranes. Absorption of drugs through the skin is proportional to their lipid solubility since the epidermis behaves like a hydrophilic barrier. Lipid insoluble drugs are therefore suspended in oily vehicles to enhance solubility and hence absorption.

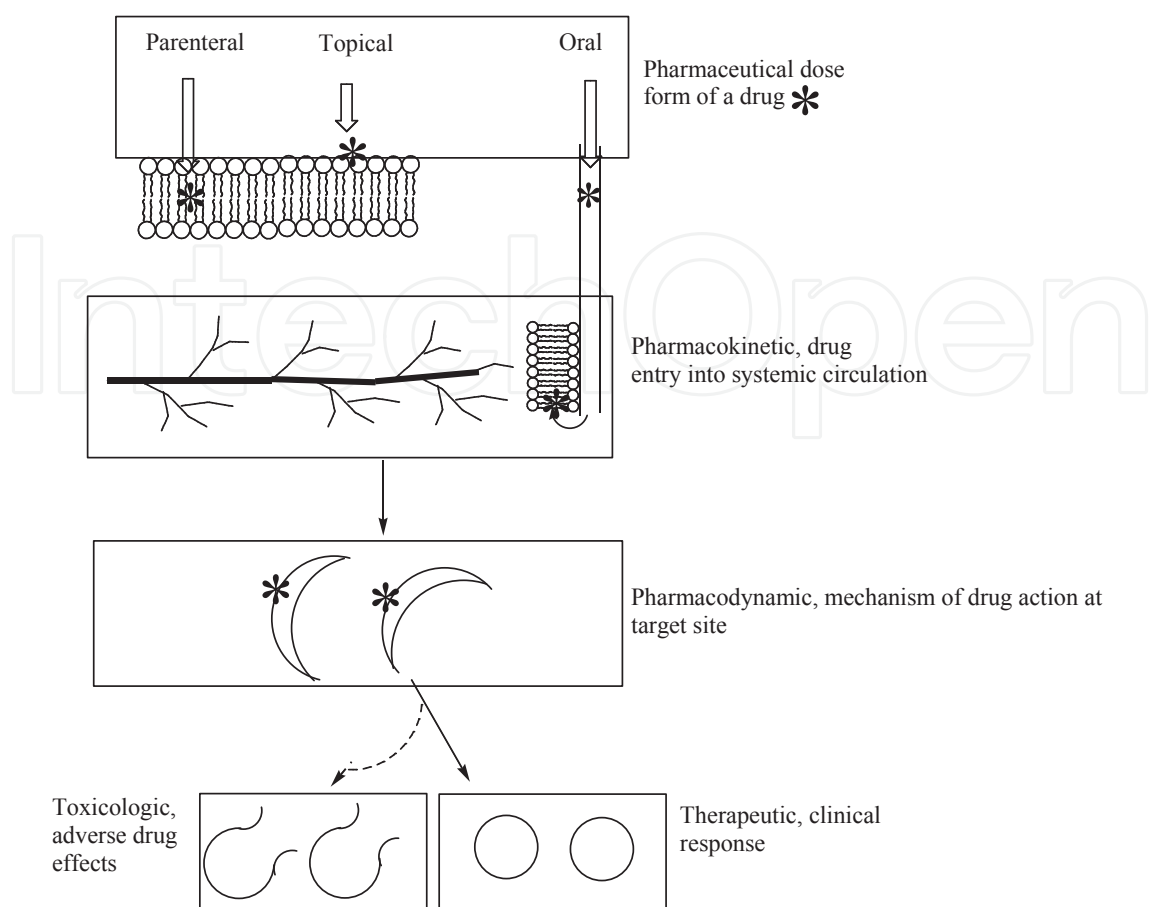


Figure 1. Relationships between the five pharmacological processes starting with the entry of the drug and ending with the clinical response and/or the toxic effects

4.2. Oral administration

The drugs administered orally are absorbed at different sites along the gastrointestinal tract (GIT):

4.2.1. Oral mucosal or sublingual

Drug absorption is generally rapid because of the rich vascular supply to the mucosa and the absence of a stratum corneum. Drugs delivered using this route are not exposed to gastric and intestinal digestive juices and are not subjected to immediate passage through the liver. Therefore there is no prior biotransformation or first-effect before the drugs enter the systemic circulation.

4.2.2. Stomach and intestine

Absorption depends on different factors such as pH, gastric emptying, intestinal motility and solubility of solid drugs. The rapidity with which a drug reaches the small intestine is enhanced when a drug is taken with water and when the stomach is relatively empty. However drugs absorbed in the stomach and intestine are subjected to first-pass effect.

4.3. Rectal administration

This is the preferred route when the oral route is unsuitable because of nausea or if the drugs have objectionable taste or odor. This route also protects susceptible drugs from the biotransformation reactions in the liver. However, absorption by this route is often irregular and incomplete. Formulations such as suppositories or enemas are applied via rectal route.

4.4. Parenteral administration

This mode of administration is also known as injection. It is generally more rapid and enables more accurate dose selection and predictable absorption. Parenteral routes include;

4.4.1. Subcutaneous injection

This mode of administration is mainly used for non-irritating drugs. It provides even and slow absorption producing sustained drug effects. Vasoconstrictor agents such as epinephrine can be added to the drug solution to decrease the rate of absorption.

Large volumes of drugs may, however, be painful because of tissue distention.

4.4.2. Intramuscular injection

This method of drug delivery ensures rapid absorption of the drug in aqueous solutions. Slow and even absorption is possible when drugs are suspended in oily vehicles.

4.4.3. Intravenous administration

This route ensures rapid delivery of the desired blood concentration of the drug to be obtained accurately and immediately and is the preferred route of delivery in emergency situations. Irritating drugs are delivered intravenously because the veins have low sensitivity to pain. This mode of delivery is also preferred for drug such as the barbiturates and phenytoin, anti-seizure drugs which dissolve only in rather strong alkaline solution and therefore need the blood to buffer the pH of the drug solution for better solubility. Drugs such as ethylene diamine tetra acetic acid (EDTA) for treatment of heavy-metal poisoning are given by intravenous injection or through an infusion because they are poorly absorbed in the gut. The other advantage of this mode of delivery is the avoidance of the hepatic and pulmonary first-pass effect. Generally, the properties of the drug may determine the route that must be used for reasonable efficacy.

5. Mechanisms of drug absorption across membranes

In order for drugs to elicit their pharmacological effects, they have to cross the biological membranes into systemic circulation and reach the site of action. Therefore an insight into the structure and function of the membrane leads to a better understanding of drug absorption.

Membranes are phospholipid bi-layers with interspersed integral and peripheral proteins which behave either as molecular 'gates' or 'pumps'. Molecular gates are non-specific. The intake of molecules into the cell depends on the charged groups in the pore and the size of molecule to be transported across the membrane. Molecular pumps, however, are highly specific and require energy for molecular transport. There are several mechanisms by which drugs traverse membranes to reach their intended target site and they include the following:

5.1. Simple diffusion

This involves is the passage of polar but uncharged substances across water filled channels in response to the concentration gradient. Simple diffusion is the mechanism of choice for water soluble drugs and those with low molecular weight such as the anesthetic nitrous oxide (44kD) and ethanol (46 KDa). The majority of lipid-soluble drugs permeate cell membranes by passive diffusion between the lipid molecules of the membrane. The permeation rate of a lipid soluble drug depends on the concentration of the drug, its lipid/water partition coefficient concentration of protons and the surface area of the absorbing membrane. The lipid/water partition coefficient of a drug is the principal factor determining its absorption.

The higher the value of lipid/water partition coefficient of a drug, the more rapidly it will be absorbed and vice versa. The chemical force that causes lipid-soluble drugs to move readily across membranes is termed the hydrophobic force since water molecules repels the lipid-soluble drugs. In most cases, drug absorption can be enhanced by absorption enhancers, such as fatty acids, phospholipids and muco-adhesive polymers. These compounds disrupt the lipid bilayer making it more permeable and also increase the solubility of insoluble drugs.

5.2. Facilitated diffusion

This type of diffusion is achieved by carrier molecules which combine with the drug in question to form complexes that can diffuse more rapidly across the membrane than free-drug could do alone. An example is the transport of nucleotide antimetabolites used in viral or cancer chemotherapy.

5.3. Active transport of drugs

This is the transport which is linked to a source of energy. Examples of specific active transport systems are the sodium pump, which maintains high potassium and low sodium ions inside the cell relative to the external medium and the calcium pump that maintains a high concentration of calcium inside the sarcoplasmic reticulum and a low concentration around the myofibrils. Active transport of drugs across membranes have been discovered and an example is the uptake of pentazocine (a narcotic antagonist) by leukocytes which is dependent upon energy supply (glucose) and can be inhibited by cyclazocine, which competes for the same transport mechanism.

5.4. Pinocytosis and phagocytosis of drugs

Proteins, bacterial toxins and drugs with high molecular weights, (1000 KDa or more) enter cells by means of pinocytosis and endocytosis. These substances finally enter the lysosomal system.

6. Factors affecting absorption of drugs

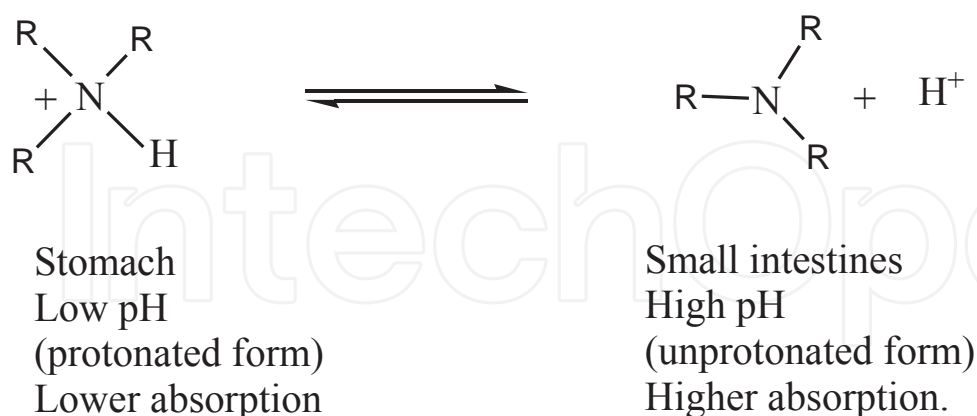
6.1. Surface area

For any substance that can penetrate the GIT in measurable amounts, the small intestine represents the greatest area of absorption. For instance, ethanol can be absorbed by the stomach, but it is absorbed eight times faster from the small intestine because of the large surface area provided by the villi. The rate at which the stomach empties its contents into the small intestine also markedly affects the overall rate at which drugs reach general circulation after oral administration. For this reason many agents are administered on an empty stomach with sufficient water to ensure their rapid passage to the small intestine.

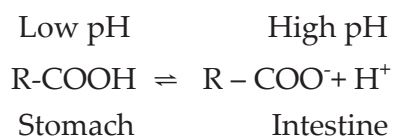
6.2. Tissue pH

Drugs can be classified either as organic amines or organic acids and therefore their absorption is markedly affected by pH. Tertiary amines are not charged at high pH and have a high lipid/water partition coefficient and hence readily penetrate membranes.

At low pH, the tertiary amine is protonated and has low lipid/water partition coefficient thus lower rate of permeation.



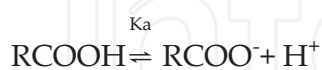
In case of an organic acid, the same general principle applies. The unprotonated organic acid at low pH permeates the tissues more readily as compared to the charged form of the drug at high pH.



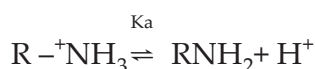
Therefore organic acids such as barbiturates and acetyl salicylic acid (aspirin) have a higher absorption rate in the stomach.

The degree of ionization of the drug when in the GIT or other body fluids is the main determinant of the amount of the drug found in an uncharged form and this depends upon the relation between pH of the fluid and the pKa of the drug:

Acidic drugs:



Basic drugs:



If the pH of the fluid is low, the ionization of acidic drugs is less while the ionization of basic drugs will be high. When the pKa of a drug is equal to the pH of the surrounding fluid, there is 50% ionization.

7. Types of tissue barriers to drugs

Most of these barriers are typically the same systems that animals use for defense against invasion by foreign agents. These barriers include the skin, the GIT membranes, blood-brain barrier and placenta.

7.1. Skin

The superficial layer of the skin, stratum corneum is particularly impermeable to most drugs. The skin permeability for the drugs is enhanced by using a co-solvent system such as ethanol/water which increases drug partition into the skin. The lipid domains of the buccal and nasal mucosa also restrict drug entry and the drugs which permeate are able to do so through passive diffusion using the hydrophilic trans-cellular spaces and direct permeation through the membrane.

7.2. Tight junctions

The gap junctions between cells in different cell types within a tissue can form channels for the passage of drugs between epithelial, endothelial, and mesothelial cells of the same tissue. These channels comprise of a group of proteins known as connexin. Cells in different tissues are however connected by tight junctions and these can impair transport between cells in different tissues. The tight junctions are dynamic structures, which normally regulate the trafficking of nutrients, medium sized compounds between cells, and form a regulated barrier in spaces between cells. There is need therefore to use drug absorption enhancers such as bile salts and long chain acyl-carnitines which act as Ca^{2+} chelators and disrupt the tight junctions thereby improving transport across the junctions. Tight junctions are shown in Figure 2.

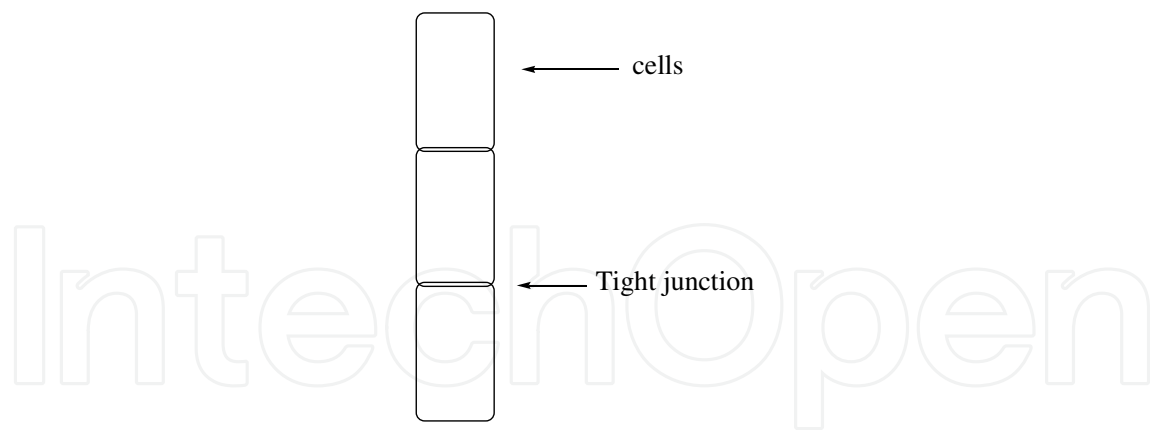


Figure 2. Arrangement of epithelial cells with tight junctions

7.3. Cerebrospinal fluid barrier (CSF)

Epithelial cells which are in contact with the brain ventricular spaces form a barrier to the movement of drugs. These epithelial cells are connected by occluding zonulae (blood- brain barrier) as shown in Figure 3. The zonulae severely restrict the passage of most molecules between the bloodstream and the parenchyma of the central nervous system. Drug entry across this barrier is through either passive diffusion or carrier mediated transport. Only the lipid soluble drugs cross into the CSF from blood.

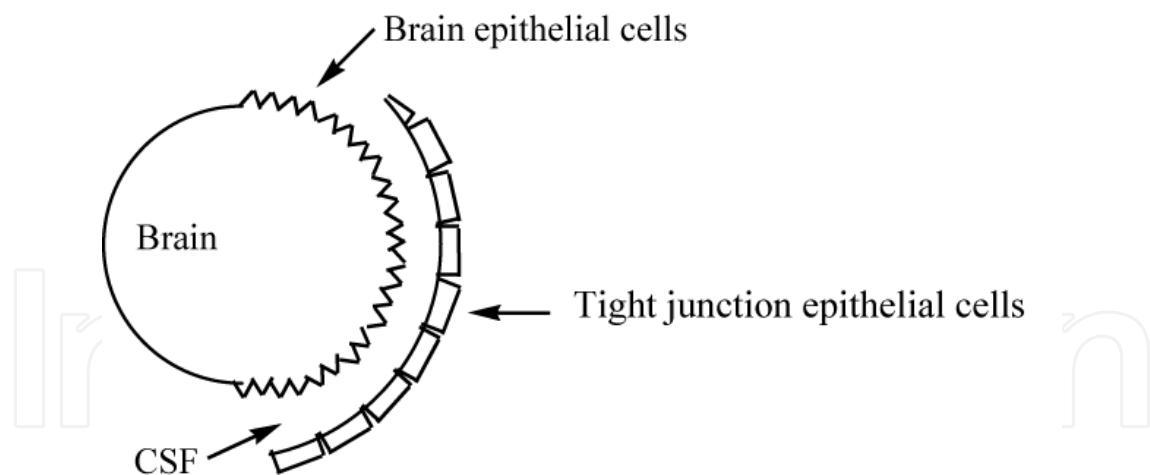


Figure 3. Epithelial cells with tight junctions as part of the blood brain barrier

Epithelial cells that separate the CSF from the brain are connected with tight junctions and are characterized by marked scarcity of pinocytotic vesicles. However, the epithelial cells that line the brain are not connected by occluding zonulae and therefore, there is unrestricted passage of drug molecules from CSF to the brain. Drugs like penicillin which are not much lipid-soluble and required in high concentrations for the treatment of brain abscesses are administered through intrathecal injections directly into the CFS.

7.4. Placental barrier

The placental membrane limits the amount of maternal blood following through the placenta to the foetus and passive diffusion is the main mechanism of drug entry from the maternal blood to the foetus. The shortest time required for equilibration of a drug between mother and foetus is about ten minutes and this delay is useful as it can allow a mother to be anaesthetized during final stages of labour.

8. Systemic availability of drugs

A drug will reach systemic arterial circulation only if it is absorbed from the GIT and if it escapes metabolism in the gut, liver, and lungs. When the concentration of the drug in plasma is measured at specified time intervals, it is possible to construct concentration versus time graph and hence be able to determine the extent of drug availability as shown in Figure 4.

The availability depends on both the extent of absorption and the extent of presystemic metabolism and comprises three aspects; Peak concentration (C_{max}), Time taken to reach the peak (T_{max}) and area under the curve (AUC) as shown in Figure 4. The C_{max} and T_{max} are measures of the rate of availability while AUC is a measure of the extent of availability (i.e. proportion of the administered drug which reaches systemic circulation intact). For the three curves shown for the formulations a, b, and c; the AUC is the same, but the rate of availability is different in each case; a, has the lowest rate of availability followed by b, while c has the highest rate of availability.

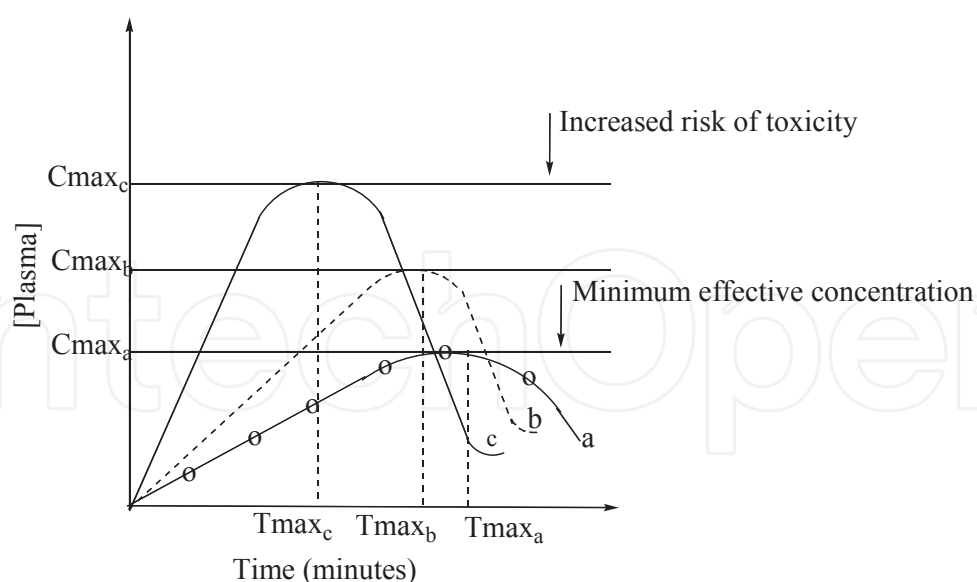


Figure 4. Plasma concentration versus time curves for different drug formulations

The speed at which a particular drug is needed to reach the site of action will determine the type of formulation to use. Drugs with the same relative bioavailability and can be used to treat the same condition using either the same routes or dosages are known as bioequivalent drugs.

9. Dosage and effect

A particular dose of an administered drug is subject to the biochemical processes in the body as shown in Figure 5. The desired effect of a drug is proportional to the concentration of the drug at its site of action which is described by the following kinetic parameters: (i) The apparent volume of distribution (V_d) which is the volume of the hydrophilic and hydrophobic spaces in the body that the drug is distributed in. It is obtained by dividing the injected dose (D_0) by the initial concentration (C_0) in blood plasma. Drugs that bind to tissues extensively exhibit low concentrations in the plasma and therefore, have higher a V_d compared to those that are mainly bound by blood plasma proteins. An average 70kg person has a total body water volume of ~ 50L of which ~ 10L occupy extra-cellular space.

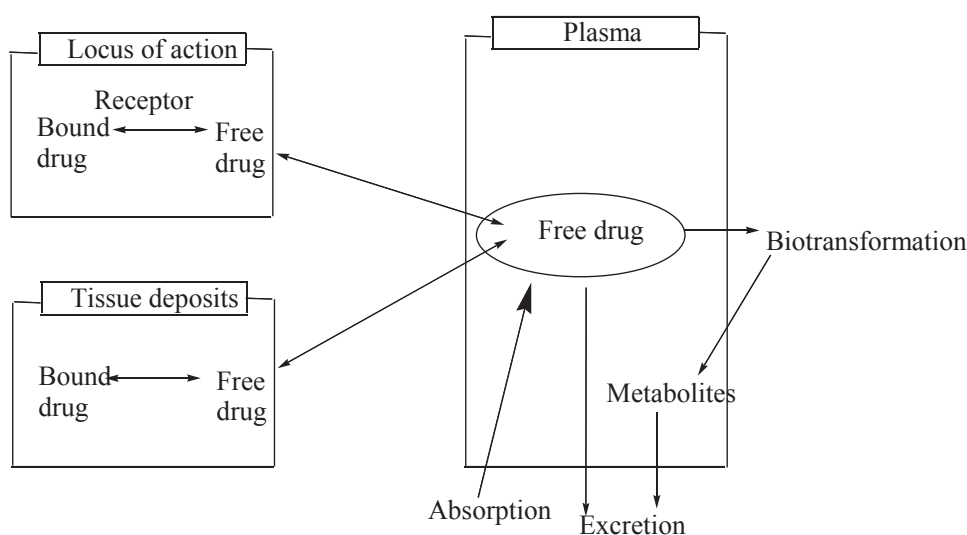


Figure 5. Drug disposition routes from absorption to excretion

The apparent volume of distribution cannot tell us where in the body the drug really is. The (C_{tox}) is the maximum drug concentration beyond which there would be toxic effects in the body, while the (C_{ther}) is the plasma concentration of a drug that would achieve a therapeutic effect or effective clinical response. The steady state concentration (C_{ss}) is that concentration that should be maintained between any two drug administration intervals. These pharmacokinetic data are important in that they characterize the fate of drugs in the body and are required by pharmacologists to calculate doses and frequencies of drug administration. However, in some clinical responses, the intensity of pharmacological action correlates better with the concentration of free drug in plasma, while in other responses there is no direct relationship between drug concentration and clinical response. The main variations of the drug response effects include;

- i. Drugs which combine with their receptors as quickly as they dissociate from them; for this category of drugs, the pharmacological effect increases or reduces in tandem with the plasma drug concentration.

- ii. Drugs which do not readily dissociate from their receptors. In this case the pharmacological effect persists despite the falling plasma concentration.
- iii. Drugs which combine with receptors and irrespective of their rates of association/dissociation sets in motion a cascade of events which runs on despite falling plasma concentrations.

10. Kinetics of drug disposition process

Drug disposition process most of the times follows the 1st order kinetics in which disposition is proportional to the concentration of the drug at any given time. Therefore, the concentration of a drug in plasma will decrease at a rate that is proportional at all times to the concentration itself. Therefore;

$$\frac{-d[C]}{dt} = K[C]$$

$$\int \frac{-d[C]}{[C]} = \int K dt$$

$$\left[\ln[C] \right]_{C_0}^{C_t} = -Kt$$

$$\ln[C]_t - \ln[C]_0 = -Kt \quad \text{or} \quad \ln C = \ln C_0 - Kt$$

$$e^{\ln \frac{[C]_t}{[C]_0}} = e^{-Kt}$$

$$[C]_t = [C]_0 e^{-Kt}$$

A more convenient form of this equation is obtained by taking \log_{10}

Since

$$\ln x = 2.303 \log_{10} x \tag{1}$$

It follows that;

$$2.303 \log_{10} C = 2.303 \log_{10} C_0 - Kt$$

$$\text{and } \log_{10} C = \log_{10} C_0 - \frac{Kt}{2.303}$$

A linear relationship is obtained when the logarithm of concentrations ($\log_{10}C$) is plotted against (t), times of observation (Figure 6).

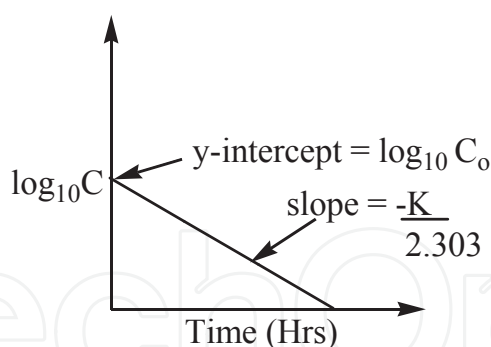


Figure 6. Logarithmic time course of drug concentration

Half-life ($t_{1/2}$) of a drug: this is the time period during which the concentration decreases to one-half of its previous value. $T_{1/2}$ can be evaluated from the elimination rate constant.

$$\text{When } t = t_{1/2}, C_t = \frac{C_0}{2}$$

therefore,

$$\frac{C_0}{2} = C_0 e^{-K t_{1/2}}$$

$$\frac{1}{2} = e^{-K t_{1/2}} \quad \text{but,} \quad K t_{1/2} = \log_e 2$$

$$t_{1/2} = \log_e \frac{2}{K} = \frac{0.693}{K}$$

11. Drug biotransformation reactions

Drugs and other foreign substances (xenobiotics) undergo series of biotransformation reactions in the body. The biotransformation reactions act as first line defense strategy against these xenobiotics. It is armed with a battery of enzymes which convert the lipid-soluble xenobiotics into more water-soluble metabolites to allow more efficient excretion of the drugs in a limited volume of water in urine or bile.

The enzymes involved in the biotransformation of endogenous chemicals are the same ones that are used in the biotransformation of xenobiotics. There is, therefore, a close relationship between drug biotransformation and fundamental homeostatic processes.

The drug biotransformation reaction may result in the following potential effects with respect to pharmacological activity:

11.1. Activation

An inactive precursor may be converted into a pharmacologically active drug. For instance, the nucleoside analogue used as an anti-HIV drug, have to undergo *in vivo* phosphorylation

to form the active triphosphates which functions to inhibit the enzyme reverse transcriptase, while L- dopa (inactive), which is used in the treatment of parkinsons disease, is converted into dopamine (active) in the basal ganglia. Futamide, a drug used in the treatment of prostate cancer, undergoes hydroxylation at the alkyl side chain to form hydroxyflutamide, a metabolite that is more active and has a longer duration of action compared to the parent drug.

11.2. Maintenance of activity

An active drug is converted into another form which is also active, for instance diazepam, a sedative hypnotic, is metabolized to an equally active metabolite, oxazepam.

11.3. Inactivation

An active drug is converted to inactive products, for example, pentobarbital is hydroxylated to form inactive metabolites.

11.4. Phase I reactions

These include oxidation, reduction and hydrolytic reactions and such reactions generally introduce or unmask a functional group (hydroxyl, amine, sulfhydryl etc) that make the drug more polar.

11.5. Phase II reactions

Consist of synthetic/conjugation reactions in which an endogenous substance such as glucuronic acid or glutathione combines with the functional group derived from phase I reactions to produce a highly polar drug conjugate. All tissues have some ability to carry out drug biotransformation reactions but the most important organs of biotransformation include; the liver, GIT, lungs, skin, and kidneys in that order and most phase II reactions result in a decrease in the pharmacological activity of the drug. The fact that the GIT and liver are the major sites of drug biotransformation means that drugs which are administered orally will be extensively bio-transformed before they eventually reach systemic circulation. This first-pass effect can severely limit the oral bio- availability of some drugs. In addition, intestinal micro-organisms are capable of catalyzing drug biotransformation reactions e.g. a glucuronide conjugate of a drug may be excreted through the intestine via the bile where gut bacteria may convert the conjugate back into free drug. The free drug is then reabsorbed and re-enters the liver via the portal vein where the conjugation process is repeated. This leads to a phenomenon known as entero-hepatic circulation.

At sub-cellular level, enzymes of drug biotransformation are located in the endoplasmic reticulum, mitochondria, cytosol and lysosome. The major site of drug biotransformation within the hepatocytes and other cells is the membrane of the smooth endoplasmic reticulum. The smooth endoplasmic reticulum constitutes the microsome fraction during differential centrifugation of whole blood. The microsome fraction can be used to carry out many drug biotransformation reactions *in vitro*.

11.6. Mechanisms of phase I reactions

11.6.1. Oxidation

Is the most important category of the microsomal drug oxidizing systems and requires participation of two distinct proteins in endoplasmic reticulum; cytochromes P_{450} (which functions as a terminal oxidase) and cytochrome P_{450} reductase. The name Cyt_{450} is derived from the fact that the reduced form of this hemoprotein complexes with carbon monoxide to form a complex that has a unique absorption spectrum with a maximum at 450nm. Cytochrome P_{450} reductase serves to transfer reducing equivalent from NADPH to the cytochrome P_{450} oxidase:



The sequence of reactions that transform a drug to its hydroxylated product is shown below (Figure 7).

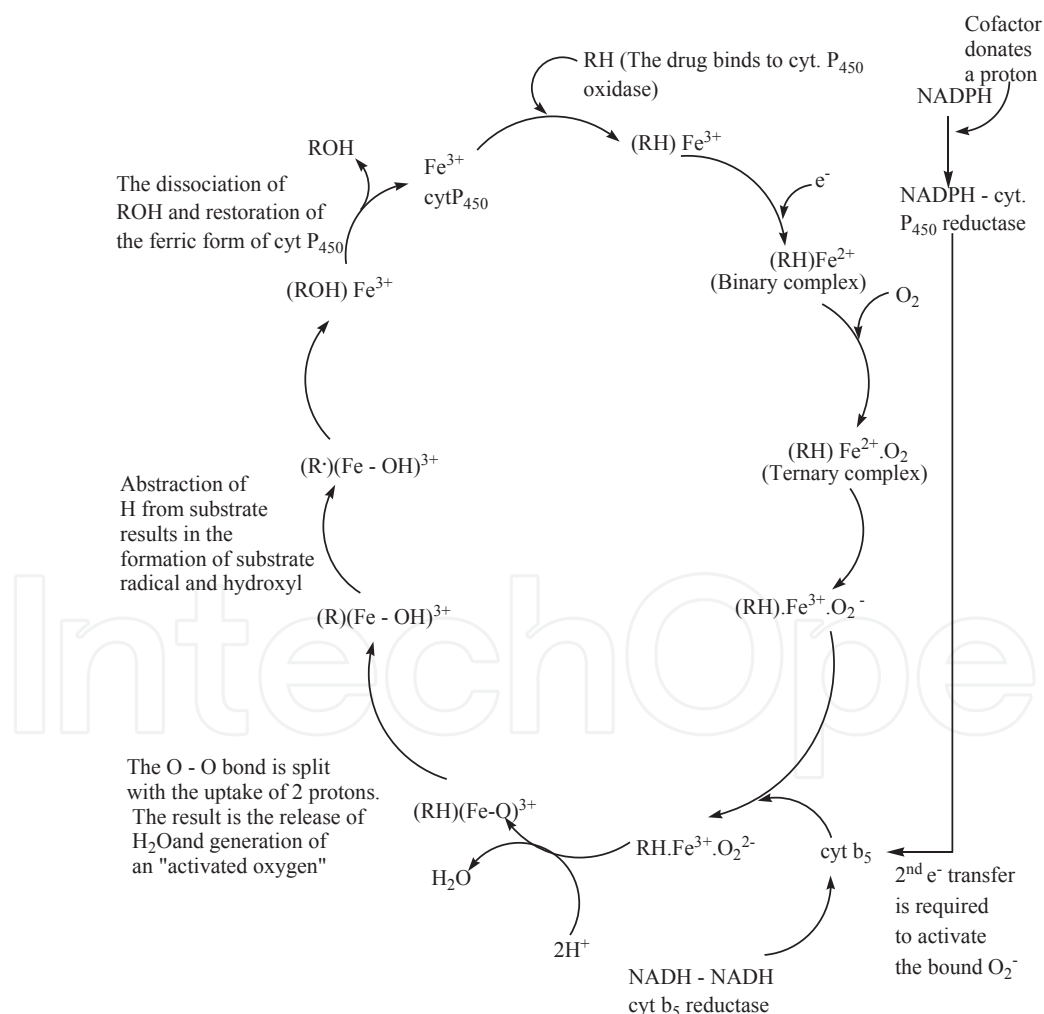
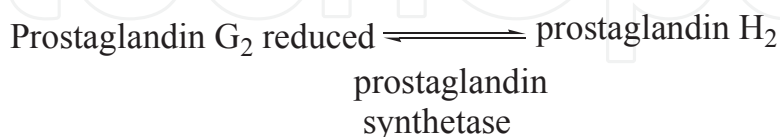


Figure 7. Phase I drug biotransformation reactions in the liver microsomal fraction in which the drug is converted to a more polar form.

The phospholipids of the endoplasmic reticulum are required for substrate binding, electron transfer, and facilitating the interaction between CytP₄₅₀ and its reductase. However, cytochrome P₄₅₀ does not catalyze all Oxidation reactions. The microsomal flavin- containing monooxygenases (FMOs) catalyze NADPH – dependent oxygenation of nucleophilic phosphorous, nitrogen and sulfur atoms. These atoms are present in a wide variety of xenobiotics including the carbamate containing pesticides and therapeutic agents such as phenothiazines, ephedrine and N-methylamphetamine. Another important drug-oxidizing system is the prostaglandin synthetase-dependent co-oxidation.



Many xenobiotics including phenytoin can be co-oxidized along with the above reduction reaction. This pathway is of considerable toxicological importance as it often leads to generation of toxic reactive metabolites. Other enzymes that catalyze oxidation of xenobiotic include alcohol dehydrogenase, aldehyde dehydrogenase, xanthine oxidase and monoamine oxidase.

11.6.2. Reduction

Some drugs with azo-linkages (RN=NR, e.g. prontosil) and nitrogen groups (RNO₂, such as chloramphenicol) are transformed by reductive pathways. The Cyt P₄₅₀ and NADPH-cyt P₄₅₀ reductase enzymes that catalyze oxidation reactions are also involved in reduction reactions for drugs containing quinone moieties. These transformation results in the formation of semiquinone free radicals illustrated in Figure 8. The free radicals that are generated cause oxidative stress, lipid peroxidation, DNA damage, and hence cytotoxicity. These effects are particularly responsible for the antitumor property of a drug like doxorubicin.

11.6.3. Hydrolysis

Drugs containing ester functions (R₁COOR₂) such as procaine are hydrolyzed by a variety of non-specific esterases in liver, and plasma while drugs with amide bonds are hydrolyzed by amidases in the liver. The polypeptide drugs such as insulin and growth hormones are hydrolyzed by peptidases in the plasma and erythrocytes. The metabolites resulting from hydrolysis reactions are subjected to phase II biotransformation reactions before excretion in the bile or urine.

11.7. Mechanisms of phase II reactions

The phase II reactions generally involve coupling of drug/drug metabolite with an endogenous substance to enhance their removal from the body. They require participation of specific transferase enzymes and high energy activated endogenous substances.

Most of the conjugation reactions result in detoxification of the drug although in some cases conjugation reactions result in bioactivation of drugs. The following is a summary of the different types of phase II biotransformation reactions;

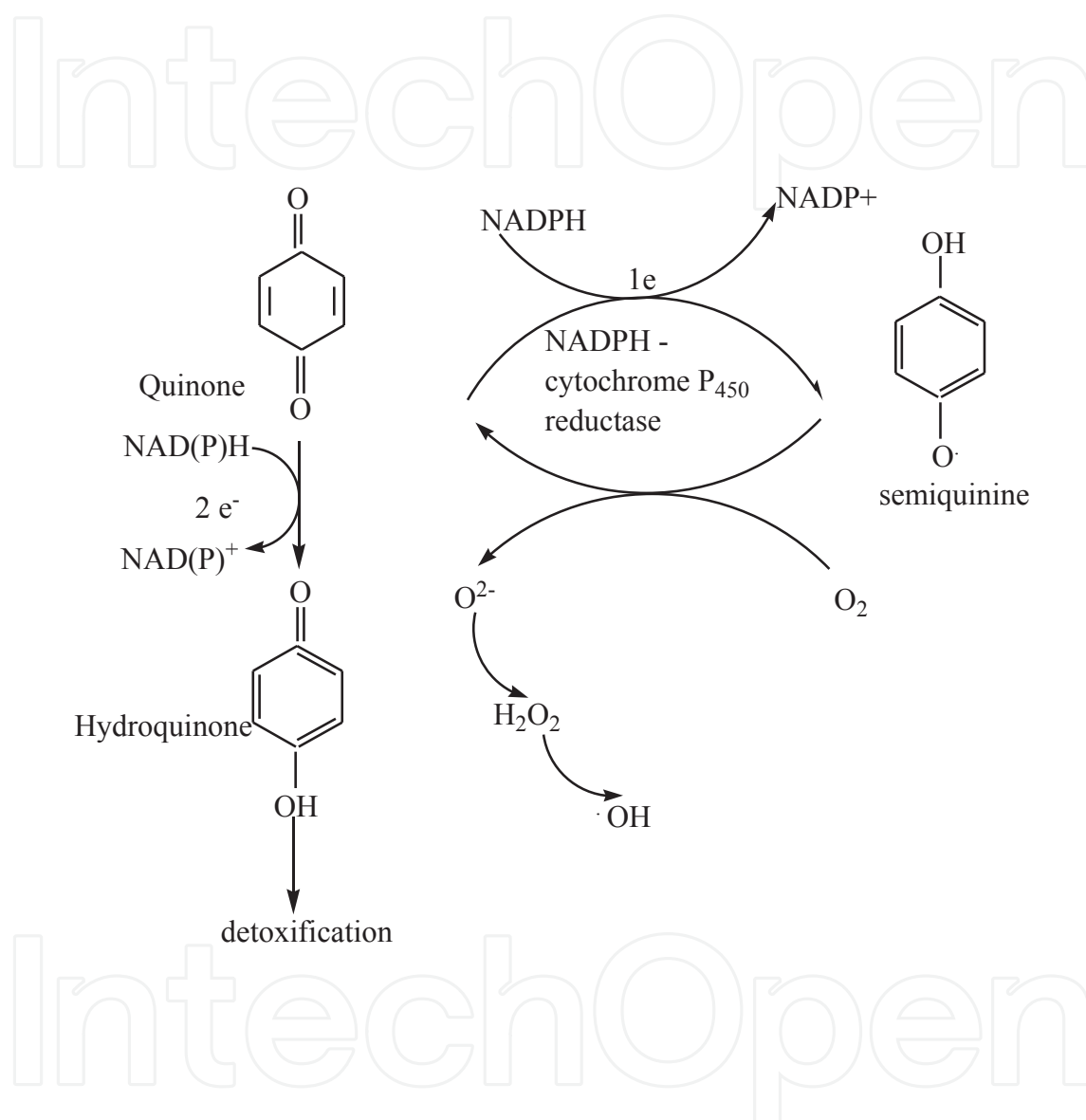


Figure 8. The transformation pathways for drugs with quinone moieties to generate free radicals.

11.7.1. Glutathione conjugation

Glutathione-S-transferases catalyze the enzymatic conjugation of xenobiotics with the endogenous tripeptide glutathione, glutamylcystenylglycine (GSH). The xenobiotics with suitable electrophilic centres such as the epoxides and nitro groups can be subjected to nucleophilic attack by glutathione (Figure 9). The final product, mercapturic acid is easily excreted from the body.

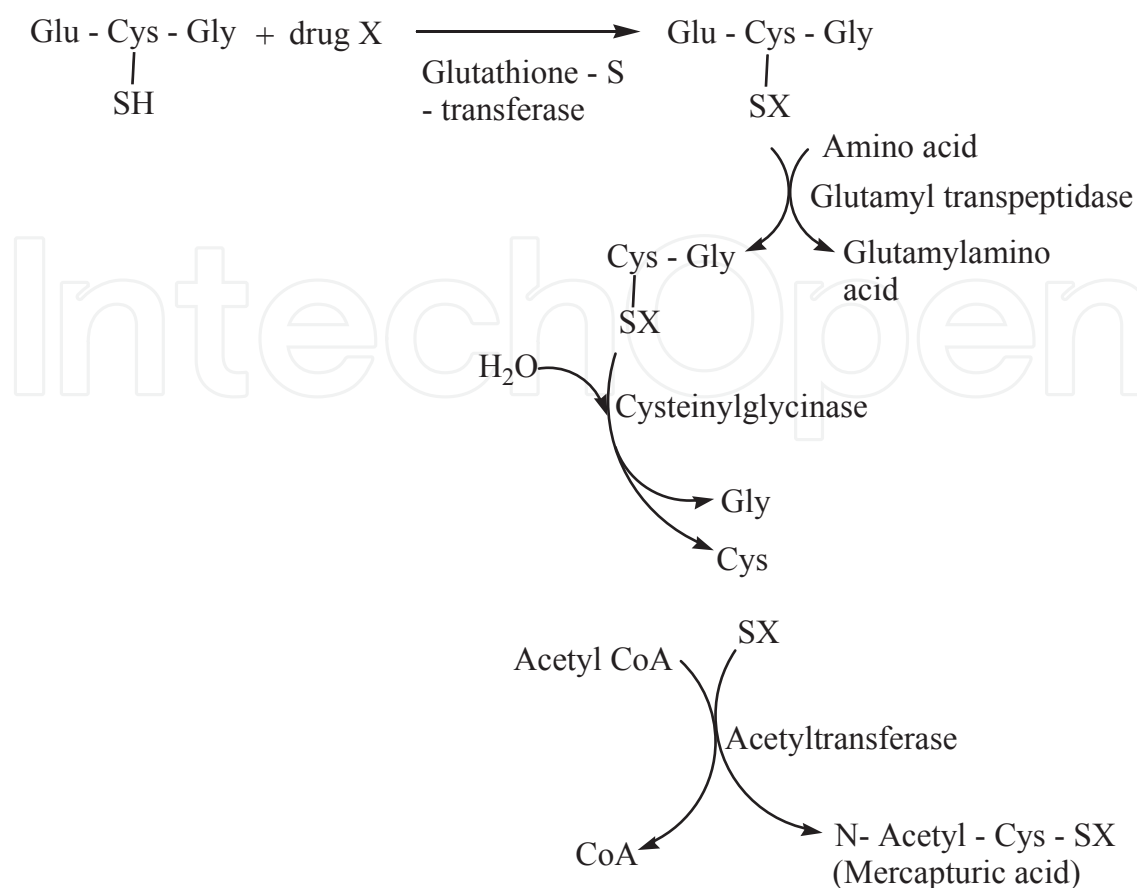
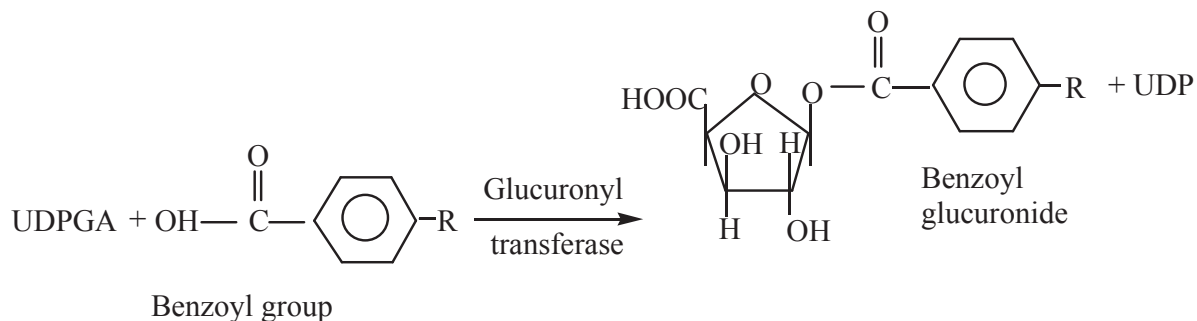


Figure 9. Glutathione conjugation reactions for a drug with a suitable nucleophilic centre leads to the formation of mercapturic acid which is easily excreted from the body

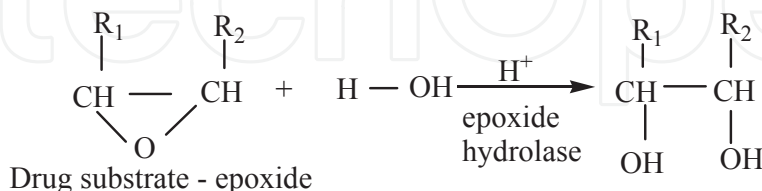
11.7.2. Glucuronidation

This is the conjugation of a drug or xenobiotic with glucuronic acid. Many functional groups are subject to glucuronidation. The benzoyl group in morphine, (an analgesic) and the amine group in meprobamate (a sedative) can undergo glucuronidation. A drug with a benzoyl group can undergo glucuronidation by a transferase as shown below:



11.7.3. Epoxide hydration

A number of aromatic compounds are transformed by phase I reactions to form epoxide intermediates. The epoxides are reactive electrophilic species that can bind covalently to proteins and nucleic acids to bring about toxic effects. These epoxides are detoxified via the nucleophilic attack of water molecule on one of the electron deficient carbon atoms of the oxirane ring as shown below:



The glucuronide conjugates can be excreted via the bile or urine.

11.7.4. Acetylation

Acetylation is achieved by cytosolic enzymes known as N-acetyl transferases which catalyze transfer of acetate from acetyl co-enzyme A to primary aromatic amine or hydrazides (figure 10)

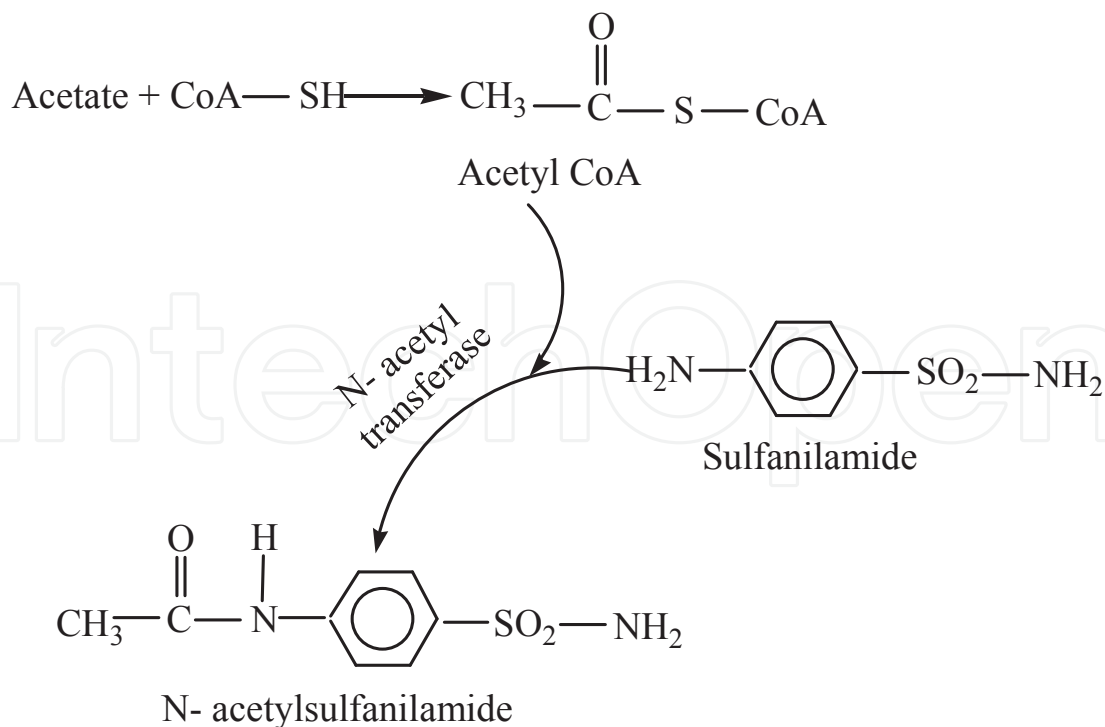


Figure 10. Acetylation reactions leading to the formation of N-Acetylsulfanilamide, the final metabolite of the antimicrobial agent sulfanilamide which is secreted from the body.

11.7.5. Methylation

Most of the methyl transferases are cytosolic enzymes. They utilize S-adenosyl methionine (SAM) as the methyl donor. The final metabolite, thiopurine, has antineoplastic properties and is used as an anticancer agent (Figure 11)

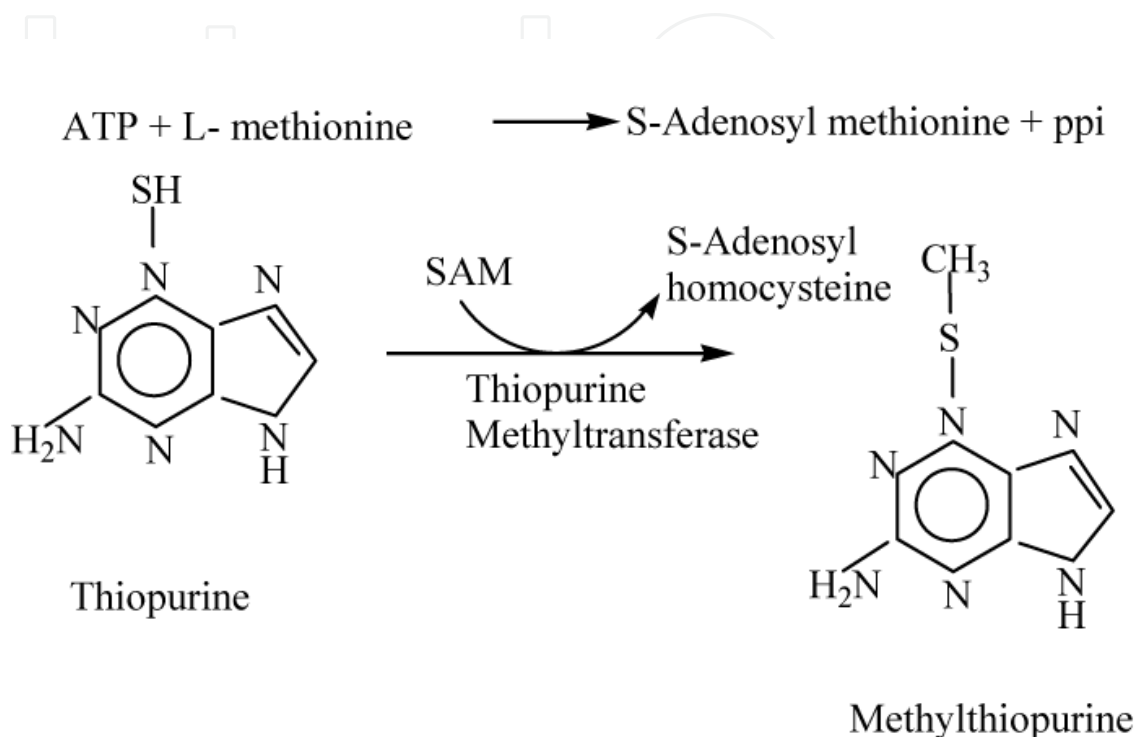


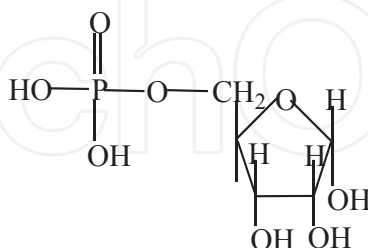
Figure 11. Methylation reactions leading to the formation of methylthiopurine

12. Adverse drug reactions associated with drug biotransformation reactions

Many adverse drug reactions can be traced to an improper balance between bioactivation and detoxification reactions. For example, when the analgesic acetaminophen is given at normal therapeutic doses, it undergoes glucuronidation and sulfation reactions that terminate the action of the drug and hasten its elimination. However, some of the drug is bioactivated via Cyt P₄₅₀ to form N-acetylbenzoquinimine, a reactive intermediate that can be detoxified by conjugation with glutathione (GSH). When excessive doses of the drug are given, glucuronidation and sulfation reactions become saturated and more acetaminophen is bioactivated via Cyt P₄₅₀. This imbalance leads to high concentrations of N-acetylbenzoquinone which cannot be sufficiently eliminated by the limited concentrations of glutathione. This metabolite binds covalently to cellular protein thiols and initiates hepatotoxicity leading to hepatic necrosis.

12.1. Revision exercise 1

1. Discuss the absorption of α -D-Ribose-5-phosphate, given that the two ionizable hydroxyl groups of the monophosphate ester ribose have pKa values of 1.2. and 6.6. The fully protonated form of α -D-ribose 5-phosphate has the following structure;



2. Using specific examples of drugs, justify their various routes of administration.

12.2. Revision exercise 2

If the concentration of a drug in plasma decreases at a rate that is proportional to its initial concentration, give an expression that describes this relationship and hence show that $[C_t] = [C_0].e^{-kt}$

12.3. Worked examples

Problem 1: Describe how you can determine the partition coefficient of a labeled drug

Solution: The partition coefficient of a drug is its differential distribution between the hydrophobic and hydrophilic phases. The distribution of the drug between these two phases can be determined by allowing equilibration of a radioactively labeled drug between aqueous buffer containing the drug and a cell membrane preparation obtained by homogenization and fractionation of a tissue sample. The ratio of drug concentration in the membrane to the concentration in the aqueous phase gives the partition coefficient.

Problem 2: Describe how you can demonstrate transport across membranes

Solution: Erythrocyte 'ghosts' or self sealing micelles formed when the erythrocytes release cytoplasmic contents upon exposure into a hypotonic solution can be used to study the uptake or release of labeled molecules across erythrocytes. When the ghosts are prepared in ^{14}C glucose, it will be possible to monitor the rate of release or uptake of the labeled ^{14}C glucose from the membranes into the aqueous environment. These 'ghosts' can also be used to study the uptake of various molecules at various concentrations under different conditions of temperatures and the presence of inhibitors for specific molecular uptake.

Problem 3: A 120mg per kg dose of a drug was injected intravenously and its concentration (mg/L) monitored regularly over time. When $\log_{10} C$ was plotted vs time (h) a linear response was obtained with a slope of -0.08 and an extrapolated y-intercept of 1.3. Calculate the following pharmacokinetic parameters;

- i. elimination rate constant (k)
- ii. initial concentration of the drug in blood plasma (C_o)
- iii. volume of distribution (V_d)
- iv. half-life of drug elimination ($t_{1/2}$).

Solution:

- i. Slope = $-k/2.303$, therefore, $k = -2.303 \times -0.08 = 0.184$
- ii. $C_o = \text{Antilog of } 1.3 = 10^{1.3} = 20 \text{ mg/L}$
- iii. Volume of distribution (V_d) = $D_o/C_o = 120/20 = 6 \text{ L}$
- iv. The half-life of elimination $t_{1/2} = 0.693/k = 0.693/0.184 = 3.7 \text{ hours}$.

Once the apparent volume of distribution is known for a particular drug the amount of drug that must be given to achieve a desired concentration can be determined from

$$D_o = C_o \cdot V_d$$

Problem 4: You have been given the following data based on a 65 kg patient; $t_{1/2}$ of drug X = 4.5hrs, $V_d = 0.56\text{L/kg}$, C_{\min} the = 5mg/L, $C_{\text{tox}} = 20\text{mg/L}$ and $C_{\text{ss}} = 10\text{mg/L}$; calculate:-

- i. Drug clearance from the body
- ii. Average rate of drug intake (Dosing rate)
- iii. Maintenance dose
- iv. Maintenance interval
- v. Initial loading dose
- vi. Loading dose at steady state concentration

Solution

Since $t_{1/2} = 0.693/k$; $k = 0.693/t_{1/2}$ i.e. $0.693/4.5 = 0.154$, and $V = 0.56 \times 65 = 36.4$.

- i. Total drug clearance = $kV = 0.154 \times 36.4 = 5.6\text{L/h} = 93.3\text{ml/min}$.
- ii. Average rate of drug intake = rate elimination constant

$$= k \times V \times C_{\text{ss}}$$

$$= 0.154 \times 36.4 \times 10$$

$$= 56 \text{ mg/h}$$

- iii. Maintenance dose

$$= (C_{\text{tox}} - C_{\text{ther}}) \cdot V$$

$$= (20 - 5) \times 36.4 = 546 \text{ mg}$$

iv. Maintenance interval = maintenance dose/ rate of elimination

$$= 546/56$$

$$= 9.75 \text{ hrs}$$

For a practical loading schedule, the maintenance interval should be lowered to say 8.0 hrs and the maintenance dose reduced proportionately: $= 546 \times 8/9.75 \sim 437 \text{ mg}$.

v. The initial loading dose

$$= C_{\text{tox.}} \cdot V$$

$$= 36.4 \times 20$$

$$= 728 \text{ mg}$$

vi. The loading dose at steady state

$$= C_{\text{ss}} \times V$$

$$= 36.4 \times 10 = 364 \text{ mg}$$

Practical problem 1

The analytical method of assaying paracetamol relies on the introduction of a nitro group into the molecule after the removal of plasma proteins through precipitation. The resultant nitrophenol compound which is formed has a deep yellow colour in an alkaline medium and absorbs at 430nm Figure 12.

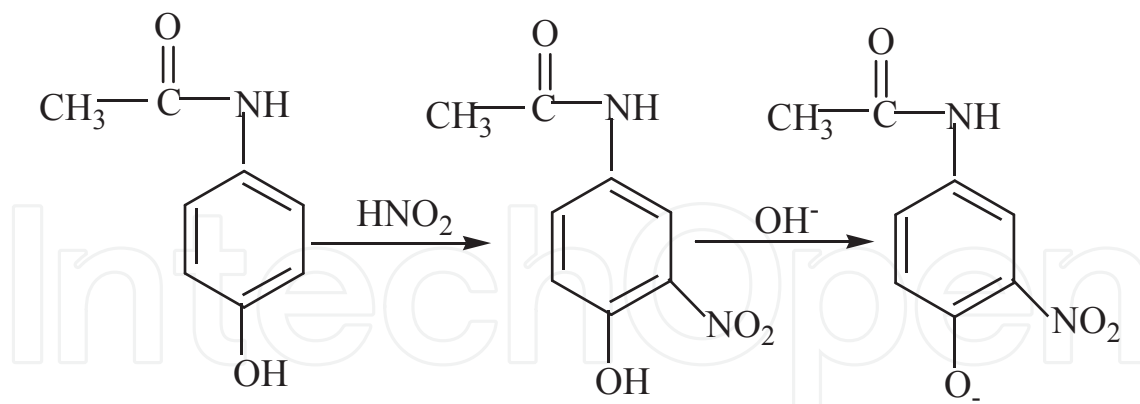


Figure 12. Formation of a chromogenic nitro compound from an analgesic Acetaminophen

i. Describe how you would construct the standard curve for determination of paracetamol concentration.

$$K = \ln \frac{x_1}{x} \frac{1}{t_1 - t_2}$$

2. Design an experiment that would enable you to determine the $t_{1/2}$ of paracetamol

Practical problem 2

Liver damage can be induced by 20% w/v carbon tetrachloride. Given 10mg/ml pentobarbitone and 10mg/ml Phenolbarbitone, design an experiment that demonstrates that the duration of action of short acting barbiturates are dependent on the integrity of the liver.

13. Drug discovery and preclinical trials

The development of new drugs over the past 30 years has revolutionized the practice of medicine and has for instance seen the increased use of new anti-hypertensives and drugs that reduce cholesterol synthesis or dissolve blood clots which led to a 50% reduction in the number of deaths from cardio-vascular diseases and stroke among other diseases.

13.1. Conventional approaches to drug discovery

These are the classical approaches to drug discovery that do not initially involve detailed scientific study they include the following;

Traditional knowledge approach

This is the discovery of drugs based on traditional medical knowledge. The best example is the documented analgesic effects of extracts from opium poppy that led to the isolation of morphine from the plant and the subsequent synthesis of related analgesics.

Discovery through serendipity

This is the accidental discovery of novel drugs based on the ingenuity of a scientist investigating a problem initially unrelated to the observed phenomenon; examples of such discoveries include the observation by Alexander Flemmings that penicilliummould could inhibit the growth of bacteria. This finding led to the discovery of antibiotics.

Discovery of therapeutic usefulness of a side effect e.g. clonidine originally used as a nasal decongestant was found to have antihypertensive properties while, the hypoglycemic effects of sulphonamides used in the treatment of typhoid fever led to the development of structurally related sulphonylureas as oral hypoglycemic drugs.

Discovery from effects of endogenous agents in test animals

An example of discovery arising from studies of endogenous agents in test animals is the anticoagulant action of the venom from the Malayan viper that led to the identification of the anticoagulant ancrod.

Modern approaches to drug discovery

These are those approaches that form a basis for the rational design of drugs and include the following;

Bioprospecting

This is the screening of a large number of natural products, chemical entities, large libraries of peptides, nucleic acids and other organic molecules for biological activity. This approach may lead to identification and development of new drug molecules.

Metabolomics

This is the profiling of natural products of related plant species screening using either liquid or gas chromatography mass spectrometry to determine active metabolites that may be present in novel crude herbal medical preparations.

In silico screening

This is the most advanced technique for drug discovery. It entails virtual screening or docking of compounds on the 3-D- structure of a known receptor based on homologies of the test drug molecules with a known test parent drug. *In silico* screening can form a basis for the modification of a known drug molecule to determine possible therapeutic applications and may lead to the development of putative drugs against new targets.

13.2. Screening of putative drug molecules

Selection of molecules for further study is usually conducted in animal models of human disease and the pharmacological tests include both the *in vitro* and *in vivo* studies after the initial screening for biological activity. For instance, antibacterial activity of drugs is assessed by their ability to inhibit growth of a variety of micro-organisms, while hypoglycemic drugs are tested for their ability to lower blood pressure.

The *in vitro* methods include incubation of a parent compound with various subcellular fractions such as microsomes, individual recombinant drug metabolizing enzymes from cells or tissue slices. The *in vivo* studies involve working on typical animal models such as dogs or rats. Some of the *in vitro* and *in vivo* studies that may be performed are shown in tables 1 and 2 below;

	Target	Specific or tissue <i>in vitro</i> studies	Biochemical measurement
a.	(i) Receptor binding	Cell membrane fraction / cloned receptors	Receptor affinity
	(ii) Receptor activity	Sympathetic nerves	Inhibition of nerve activity
	(iii) Enzyme activity e.g. tyrosine hydroxylase	Purified enzymes from adrenal glands	Inhibition of enzyme activity
b.	Cellular function	Cultured cells	Cell viability
c.	Isolated tissue	Blood vessels, heart lung or ileum from rat	Effects on vascular contraction and relaxation

Table 1. Screening of drugs for specific inhibitory effects on enzymes and isolated tissues.

	Disease model	Animal model	Route of administration	Physiological measurements
a.	Blood pressure	Hypertensive rat (conscious)	Parenteral	Systolic/diastolic
b.	Cardiac effects	Dog (conscious)	Oral	Electrocardiography (cardiac output)
		Dog (anesthetized)	Parenteral	
c.	CNS	Mouse, rat	Parenteral	Degree of sedation
d.	Respiratory effects	Dog/guinea pig	Parenteral	Respiratory rate and amplitude
e.	GIT effects	Rat	Oral	GIT motility and secretions

Table 2. Putative animal models used in studying effects of drugs

If an agent possesses useful activity it would be further studied for possible adverse effects on other major organs. These studies might suggest the need for further chemical modification to achieve desirable pharmacokinetic/pharmacodynamic properties.

13.3. Preclinical trials

The data from animal studies form a basis for the calculation of the initial or starting doses to be used in the subsequent clinical studies. The human equivalent dose calculations for the maximum recommended dose are normally based on either the body surface area or body weight. The candidate drugs that survive initial screening and profiling must be carefully evaluated for potential risks before and during clinical testing. The main types of evaluation needed from safety and toxicity studies include:-

Acute toxicity

This involves looking at the effects of large single doses of therapeutic agent. Acute toxicity studies are usually performed in animal models such as mice and rats. These studies enable investigators to correlate any observed effects with the systemic level of the drug.

Sub-acute toxicity

This is similar to acute toxicity but measures the effects of multiple doses based on expected duration of clinical usage. It entails haematological, histology and electron microscope studies to identify organs which might be affected by toxicity. It usually lasts between one to three months. This enables the selection of putative compounds for subsequent studies.

Chronic toxicity testing

These studies are required when the drug is intended to be used in humans for prolonged periods. The goals of this investigation are mostly similar to those of sub-acute toxicity.

The reproductive performance

These are measurements intended to determine the effects of the drug agents on; mating behaviour, reproduction, parturition, progeny birth defects, and postnatal development.

Carcinogenicity studies

These studies are required to determine the effects of prolonged usage of the drug under investigation. They involve hematological and histological autopsy analysis.

Mutagenicity studies

These studies look at the genetic stability and mutations of bacterial or mammalian cells in culture. These studies are at the academic research level and are intended to provide data for future research.

Investigative toxicology

The main purpose of toxicology is to discover the pathways that are involved in toxic action. It includes studies on mechanisms of toxic action of drugs which may lead to the development of safer drugs.

14. Evaluation of new drugs and drug approval process

Toxicity testing is time consuming and expensive and may require two to five years to collect and analyze data before the drug can be considered ready for testing in humans.

Large numbers of animals are needed to obtain valid preclinical data.

Extrapolation of toxicity data from animals to humans may not be completely reliable.

The safety or efficacy of a drug must be thoroughly understood before the drug is administered to any group of individuals. Therefore regulations governing the development of new drugs have evolved to assure safety and efficacy of new medications. The clinical trials during drug development and post marketing experience form the scientific basis of patient response to a drug.

Once a drug is judged ready to be studied in humans, a notice of clinical investigational exemption for a new drug (IND) must be filled with the government body concerned with the regulation and registration of drugs. The IND includes manufacturing information, all data from animal studies, clinical plans and protocols and the names and credentials of physicians who will conduct the clinical trials.

14.1. Phase I clinical trials

The main goal in phase I is to determine whether test animals and humans show significant different responses to the drug and to establish limits of the safe clinical dosage range. The measurements carried out in phase I include, the rate of absorption, $t_{1/2}$ and

metabolism of the candidate drug compound. The effects of the drug as a function of dosage are established in a small number 25 – 50 of healthy volunteers. When the drug is expected to have significant toxicity, as often the case with cancer and AIDS therapy, volunteer patients with the disease are used instead of the healthy volunteers. The requirements of clinical trials include the following:

- i. Homogenous populations of patients must be selected.
- ii. Appropriate controls for the investigation must be included.
- iii. Meaningful and sensitive indices for drug effects must be used i.e. well defined end-points such as survival or pain relief should be used rather than surrogate or intermediate markers e.g. levels of enzymes involved in the process of survival/pain relief.
- iv. The experimental observations must be converted to data and then into valid conclusions.
- v. The accuracy of diagnosis and severity of the disease must be comparable between the groups being contrasted.
- vi. The dosages of the drugs must be chosen and individualized in a manner that allows relative efficacy to be compared at equivalent toxicities.
- vii. Compliance with experimental regimens should be assessed before subjects are assigned to experimental or control groups. Non-compliance may cause false estimates of the true potential benefits or toxicity of a particular treatment.
- viii. Ethical considerations. These may be the major determinants of the types of controls that can be used e.g. for therapeutic trials that involve life threatening diseases for which there is already in-effective therapy, the use of a placebo is considered unethical. In such cases, new treatments must be compared with standard therapies.

14.2. Study design of phase I trials

For clinical trials to have validity they must be based on a sound statistical basis. Some of the criteria that must be met include;

Randomization

Randomization is a design which ensures that there is no bias in allocation of treatments among the different groups. The purpose of randomization is to minimize the possibility that an observed treatment effect is due to inherent differences between groups. Randomization eliminates bias by avoiding recruiting patients who have a particular characteristic to one group and not the other e.g. only women/men and smokers/alcoholics. Randomization should not be carried out until immediately before treatment. The delay allows a patient to have second thoughts about taking part or the investigator to have to re-consider about admitting patients to the study. Simple methods of randomization can be designed using published tables of random numbers, where treatments are in a form of a square in which each treatment is

contained only once in each row and column and the order of treatment is different in each group (Table 3). The presence of other diseases or risk factors should be taken into consideration i.e. need for careful selection and assignment of patients to each of the study groups.

Group Treatment				
1	A	B	C	D
2	C	D	A	B
3	D	C	B	A
4	B	A	D	C

Table 3. A random number table array for assignment of various treatment regimes to various groups of patients in clinical trials

This approach eliminates systematic variation between groups since the patients are allocated at random order to group 1, 2, 3, or 4. A code list should be drawn up so that the main investigators may be kept blind to the treatment an individual is receiving but also so that it is possible to know the treatment by breaking the code. Coding should be such that when broken, it does not yield information about the treatments other patients are getting. The treatment information about all the patients should be left to one person preferably the pharmacist or trial co-ordinator.

Blindness

Blinding is a design which does not allow the investigator to know what treatment the patient is receiving. The purpose of blinding is to eliminate bias in reporting the outcome of the treatment since if an investigator knows what treatment the patient is taking, he/she may in some way influence a measurement or an outcome thus shifting the outcome in one direction or another consciously or unconsciously. The ideal trial should be double blind where neither the investigator nor the patient knows what treatment the patient is taking. Placebos or dummies are used in order to achieve blindness. The placebos should match the active treatment as closely as possible in terms of size, shape, color, texture, weight, taste and smell with the active formulation.

Number of testing centers

Clinical trial should be carried out in a defined centre so as to minimize variations in the population and in the investigators techniques. This also avoids problems of data collection, communication and follows up. Multi-centre trials may become necessary when studying a rare disease hence scarcity of patients or when the effect being investigated is small one e.g. when one is looking out for an interaction effect between a major condition and a minor condition.

Clinical trials designed to evaluate efficacy of new drugs should always be prospective i.e. the characteristics of the population to be studied should be identified before the study begins. For example, if one randomized all patients with heart failure to treatment with either digoxin or a new drug X and then studied the outcome over six months that would be a prospective

trial. In case of control study the outcome is first identified and then comparisons are made retrospectively between the characteristics of patients who did or did not have the outcome. Such a study for instance has shown that oral anticoagulants can reduce incidences of re-infarction in patients who have already had a myocardial infarction. The case control studies may be carried out some time, after the introduction of a drug therapy in order to get some idea of its place in the overall management of the disease since the results of a case control study may prompt formal prospective trials in order to confirm original findings.

14.3. Molecular markers in drug development

In vitro predictive efficacy and toxico-genomics should be carried out after phase 1 clinical trials in order to validate the results of the phase 1 clinical trials. This is achieved by using animal cell lines in which gene expression profiling and patterns of protein production are used to identify candidate biomarkers for the disease. The utilization of markers that are associated with the disease or those that indicate a known response to a therapeutic intervention or reflect a clinical outcome may yield information on efficacy or toxicity of a test drug. An example of a biological readout that has traditionally been used to determine efficacy during the treatment of diabetics is the determination of glucose in the urine of a diabetic patient. Reliable and specific biomarkers that act as predictors of efficacy or long-term toxicity are useful because they reduce the time, size and cost of clinical trials.

14.4. Phase II clinical trials

These are studies that recruit willing and informed patients and are designed to assess long term safety, refine pharmacokinetic data, determine optimal dose. The purpose of phase II studies is to determine efficacy. Typically, phase II trials require 100-150 subjects and take 9-12 months. An assessment of no effect or no worthwhile effect of a given drug demonstrates that it is futile to proceed with further clinical testing of the drug. It is therefore important to minimize type I errors or false negatives in the study design in order to minimize the risk of discontinuing a potentially effective drug. The data from well designed phase I and phase II trials are therefore critical in planning the subsequent trials. The phase III trials are large trials intended to determine whether a treatment is effective and to establish safety data.

Phase II clinical trials include inert placebos as negative controls and older active drugs as positive controls alongside the investigative compound. These studies are done in special clinical centers such as University Hospitals. A broader range of toxicities may be detected at this phase.

14.5. Phase III clinical trials

The drug is evaluated in a much larger number of patients (thousands) to further establish safety and efficacy. Phase III trials are performed in settings similar to those anticipated for the ultimate use of the drug. After successful phase III trials, the next step is the application for review of the new drug to seek approval to use the drug for clinical management of the disease condition.

14.6. Phase IV clinical trials

This phase is concerned with post-marketing surveillance and the main goal is to assess adverse reactions, patterns of drug utilization, discovery of additional indications. The interrelationships between the various studies in drug development are illustrated in Fig 13 below;

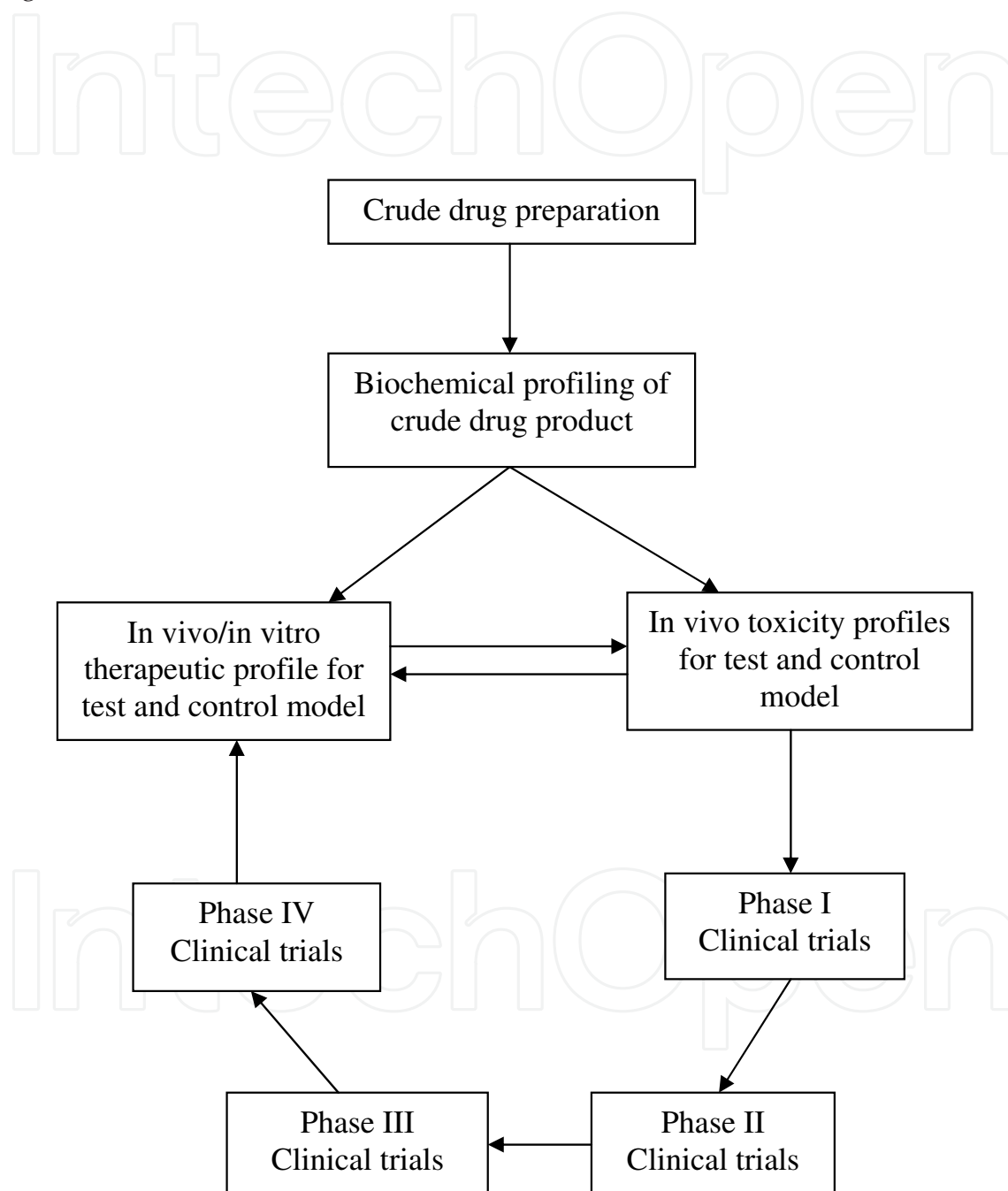


Figure 13. Illustration of the key steps in the development of a drug from a putative drug candidate extract

14.7. Pharmacogenomics and drug development

The personalized medication which takes into account the genetic make-up of individuals is known as pharmacogenomics. The pharmacogenomic differences that determine individualized therapy include genetic polymorphisms of drug transporters, drug receptors, and drug metabolizing enzymes. For example, genetic variation in Cyt P₄₅₀ enzymes that are largely responsible for drug metabolism shows that different individuals respond differently to drug efficacy or toxicity. Genetic variants in the drug target, the disease pathway, genes or drug metabolizing enzymes could all be used as predictors of drug efficacy or toxicity. For example, drug monitoring using perphenazine, a Cyt P₄₅₀ substrate, shows that there are three main categories of individuals; the efficient metabolizers obtained from the heterozygotes, the poor metabolizers from the homozygotes and the ultra-rapid metabolizers which carry two or more active genes in the same chromosome, a phenomenon known as gene duplication.

The information obtained from pharmacogenetic studies can be used to design new drugs that take the persons' genetic profile into consideration. The most common type of genetic variation are single nucleotide polymorphisms, therefore, a high resolution of single nucleotide map may expedite the identification of genes for various diseases. The molecular profiles of patients identified in phase I and II clinical trials as likely non-responders to the putative drug under investigation might present an opportunity to initiate new discovery programs for other pharmaceutical compounds.

14.8. Individualized drug therapy

Clinical usage of drugs requires a basic understanding of the pharmacokinetic and pharmacodynamic drug processes and an appreciation that a relationship does exist between the pharmacological effect or toxic response to a drug and the concentration of the drug. The interpatient and inpatient variation in disposition of a drug must be taken into account in choosing a drug regimen.

A drug dosage regimen therefore is a recipe for the administration of a drug so as to produce a desired therapeutic effect with minimum toxic effects.

The regimen is described in terms of the following:

- i. Dose of the drug to be used and the formulation.
- ii. Frequency with which it is administered.
- iii. Route of drug administration.

The factors that determine the relationship between the prescribed drug dosage and drug effect operate at three levels; prescription level, drug administration level and at the physiological level of patient (Figure 14).

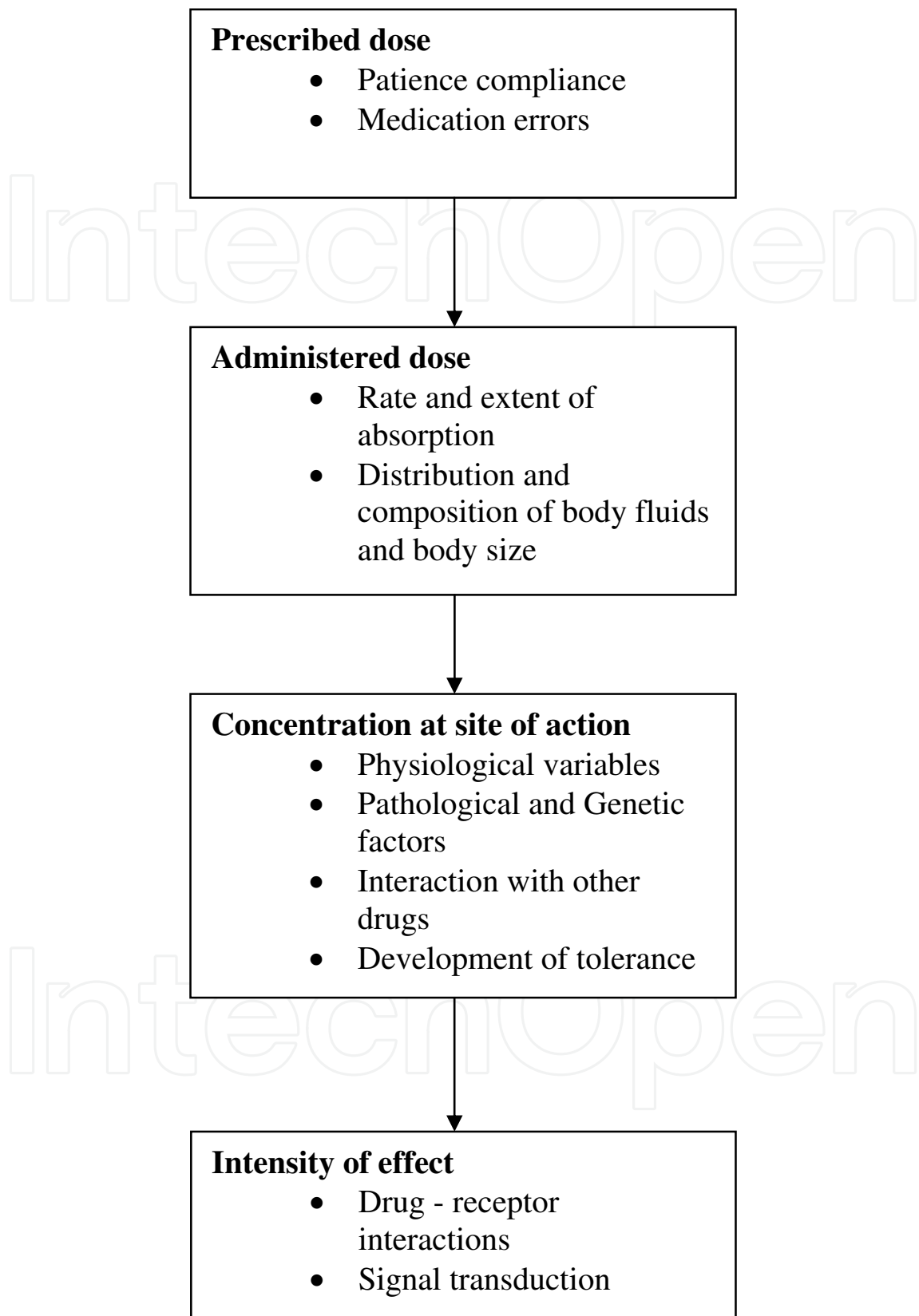


Figure 14. The operational levels that determine the relationship between prescribed drug dosage and the drug effect.

Drugs that are excreted primarily unchanged by the kidneys tend to have low variation among patients with similar renal function than do drugs which are inactivated by metabolism. For the extensively metabolized drugs, those with high metabolic clearance and large first pass elimination have marked difference in bioavailability, whereas those with low biotransformation tend to have largest variation in elimination rates among individuals.

14.9. Determination of drug dosage

The simplest way of determining a drug dosage regimen is to base it on the published recommended dosage. These are derived from the pharmacokinetics studies of the drug and the general procedure in using the published recommendations is to start at the lower end of the recommended dosage range and monitor the therapeutic effect. If the desired effect does not occur, the dosage can be increased gradually until one reaches the upper limit of the range. In certain conditions it may be necessary a sufficiently high dose for the drug to accumulate in the body to a satisfactory degree. This dose is known as the loading dose and is equal to the volume of distribution multiplied by the target concentration in the plasma. The reason for giving a loading dose is to circumvent the sometimes unacceptable time lag preceding the steady state levels. Once the correct loading dose is given, a steady-state concentration can be achieved rapidly and then maintained by giving a smaller maintenance dose.

Adjustment of dosage in individual patients is often as a result of the modification of pharmacokinetic parameters of which the three most important include; the bioavailability or the fraction of a drug that is absorbed into systemic circulation, its clearance and the volume of distribution.

For drugs with a high toxicity to therapeutic ratio, the loading dose can be given as a single dose and for drugs with a low toxicity: therapeutic ratio and a long half-life, the loading dose can be divided into several portions and given at intervals long enough to allow detection of adverse effects, but short enough to ensure that the loading dose is a true loading dose i.e. relatively little amounts of the drug is eliminated from the body during the period of loading.

14.10. Systemic drug availability

The extent of availability of a drug after oral administration is expressed as a percentage of the dose. The fractional availability (F) varies from 0 to 1. The extent of availability is more important parameter to measure rather than the rate of availability.

A true decrease in bioavailability could be due to several reasons including, a poorly administered dosage form that fails to disintegrate or dissolve in the GIT, interaction with other drugs in the GIT, metabolism of the drug in the GIT and/or first pass hepatic metabolism or biliary excretion.

Hepatic disease may in particular cause high availability because the metabolic capacity decreases or development of vascular shunts in the liver. Significantly high availability requires dosage adjustment by a factor of two, while significantly low in availability requires dosage adjustment by a factor of half.

14.11. Maintenance dose

In most clinical situations drugs are administered in such a way as to maintain a steady concentration i.e. just enough drug is given in each dose to replace the drug eliminated since the preceding dose. Therefore, clearance is the most important pharmacokinetic term to be considered in defining a rational steady-state drug dosage regimen.

The rate of elimination = $Cl \times T_c$

Where, Cl is the rate of clearance and T_c is the target concentration of the drug.

The Dosing rate = Rate of elimination = $Cl \times T_c$.

Therefore, if the target concentration is known, the prevailing clearance in that patient will determine the dosing rate and if the drug is given by a route that has a bio availability of less than 100%, then the dosing rate above can be modified using the formula:

$$\text{Dosing rate oral} = \frac{\text{Dosing rate}}{\text{Fractional availability}}$$

If intermitted doses are given, then maintenance dose = Dosing rate \times Dosing interval.

14.12. Alteration of maintenance dose

The maintenance dose is usually altered when the clearance of the drug changes. For example, during renal impairment, the clearance of drugs which are predominantly cleared by the kidney is greatly reduced and therefore, the desired steady state concentration can only be achieved either through altering the dose or altering the dosing interval. Therefore, when a drug is cleared almost completely via kidneys, the dosage interval should be changed in proportion to renal clearance as follows:

The % eliminated in dosing interval should be proportional to creatinine clearance by a published constant to yield the percentage excreted in one dosage interval.

The quantities required for this adjustment are:

- i. Fraction of normal function remaining, and the
- ii. Fraction of drug usually excreted unchanged in urine.

The fraction of normal function remaining is equal to the ratio of patient's creatinine clearance to a normal value (120 ml/min/70kg).

The following equation is for adjustment of renal clearance

$$rf_{pt} = 1 - fe_{nl}(1 - rfx_{pt})$$

Where;

rf_{pt} = the adjusted total clearance of the patient,

fe_{nl} = fraction of drug excreted unchanged in normal individuals,

rfx_{pt} = fraction of renal clearance of the normal individual.

Clearance should also be adjusted for the size of the patient and for convenience, the published values are normalized to the metabolic rate = weight^{0.75}.

When clearance is low, $t_{1/2}$ is similarly high and when the volume of distribution is high, the $t_{1/2}$ is also high. Therefore, by using parameters for the individual patient, the dosing rate = $T_c \times Cl/F$ where, T_c = target concentration, Cl = clearance and F = fractional availability of the drug.

If a drug is relatively non toxic then the maximum loading strategy can be employed so that the dosing interval is much longer than $t_{1/2}$. For example $t_{1/2}$ of penicillin is less than one hour but it is usually given in very large doses every six to twelve hours since it is non-toxic. The normal steady-state theophylline concentration can be determined using the equation:

$$C_{ss, \max} = \frac{F \cdot \text{dose} / V_{ss}}{1 - \exp(-KT)}$$

$$C_{ss, \min} = \frac{F \cdot \text{dose} / V_{ss} \cdot (\exp^{-KT})}{1 - \exp(-KT)}$$

Where, $C_{ss, \max}$ and $C_{ss, \min}$ are the maximum and minimum steady state concentrations,

$$T = \text{dosage interval and } K = \frac{0.693}{t_{1/2}}$$

14.13. Drug dosage adjustment in old patients (geriatrics)

Drug absorption in the elderly is slightly different from the normal patients and therefore adjustment of the dosage should be taken into consideration during drug therapy. The rate of transdermal drug absorption may be diminished in elderly because of reduced tissue blood perfusion. Compounds that permeate the intestinal epithelium by carrier mediated transport mechanisms may be absorbed at lower rates in the elderly.

14.14. Drug distribution

In geriatrics the 'body mass' declines with age and the total body water content falls by between 10 – 15%. The volume of distribution of hydrophilic drugs will therefore decrease while plasma concentration will increase and the likelihood of toxic drug effects will also increase. When geriatric patients use diuretics, the extracellular space reduces even further leading to a higher likelihood of drug toxicity. The total body fat in the elderly increases by 12 – 18%, therefore, for hydrophobic drugs, the higher volume of distribution implies an increase in half life of distribution and the time needed to reach steady-state serum concentration. Therefore, for geriatrics a once or twice daily drug administration is optimal. This can be achieved though delayed release or fixed drug combinations.

14.15. Patient compliance and rational use of drugs

Drug treatment of any kind is often compromised by lack of full compliance by the patient. The common errors of compliance to a regimen by a patient include; omission in taking the

drug, wrong timing of dosages, premature termination of therapy or using additional medications. In order to improve patient compliance, the patient should be made to understand the nature and prognosis of the illness and what to expect from the medication by detailing both the acceptable and undesirable unwanted effects as well as signs of efficacy that may help enforce compliances.

Patients frequently discontinue taking a medication such as septrin because they have not been told the necessity of continuing with the drug after the acute symptoms have subsided.

The effectiveness of physician-patient communication is inversely related to the error rate in the taking of drugs. A physician might prescribe a drug to be taken three times a day with meals for a patient who either eats only twice a day or sleeps all day and works at night. Therefore, an exploration of the patients eating, sleeping and working habits is necessary before a prescription is given.

The educational level of a patient may also require that the prescription is carefully worded and oral instructions given in the primary language of the patient since when such patients take three or more medications they are less likely to use them properly. It is therefore important to provide identifying symbols for each medication e.g. "Heart pill" or "sugar pill" and to reduce the doses into once or twice daily regimens.

14.16. Adverse drug reactions

Pharmacological formulations are potentially harmful to the individuals taking the drugs. There is need to ascertain the safety of new drugs before allowing them to be marketed. The following figures highlight the magnitude of the problem: ~ 10 – 20% of hospitalized patients suffer adverse drug reactions, while 0.3 – 5.0% inpatients admissions and ~ 0.3% deaths in hospital are due to adverse drug reactions. Adverse drug reactions can be classified into two main categories: They may be dose related or non dose related with each being short-term or long-term.

14.17. Dose related adverse reactions

Adverse drug reactions can occur because of the changes in the systemic availability of a formulation. For instance, the change of excipients in phenytoin capsules from CaSO_4 to lactose leads to high availability and hence adverse drug reactions. Sometimes, adverse drug reactions can occur due to the presence of contaminants like bacteria if quality control breaks down. Out-of-date formulations may also cause adverse drug reactions because of degradation products arising from the drug e.g. outdated tetracycline may cause Faconis Syndrome (a type of rickets) because of the transformation product, epiandrotetracycline. Dose related adverse reactions may also arise from pharmacokinetic variations in the individuals taking the drug. Pharmacokinetic variations may also arise due to hepatic disease like advanced cirrhosis which lowers the clearance of drugs such as phenytoin and morphine. The pharmacological variations could be environmental such as diet or smoking, while others are genetic.

14.18. Non-dose related adverse drug reactions

These include immunologic reactions and are related to the surface proteins present on β -humans lymphocytes (HLA antigens) which are important in the function of T-lymphocytes. The association of HLA antigens with foreign antigens stimulates T-lymphocytes. Some of these antigens expressed by major histocompatibility complex (MHC) genes have been associated with an increased risk of adverse drugs e.g. nephrotoxicity from penicillamine is increased in patients with HLA types B8 and DR 3 while patients with HLA-DR, 7 are protected against adverse drug effects.

14.19. Types of drug allergies

Drug allergies can be classified into five categories:

Type I

This anaphylaxis or immediate hypersensitivity reactions; the body reacts within five to thirty minutes. The IgE molecules fixed to mast cells and basophil leucocytes release histamine and other pharmacological mediators such as kinins. Drugs likely to cause are anaphylactic shock include;-penicillins, streptomycin, local anaesthetics etc.

Type II

In these reactions, the circulating antibody of IgG, IgM or IgA interacts with the drug combined with a cell membrane protein to form a hapten-protein/antigen-Ab complex. This leads to the activation of the complement leading to cell lysis of phagocytic attack of the cell with the complex. Drugs such as the cephalosporins, penicillins, quinine and transfusion of improperly matched blood can yield this type of reactions.

Type III

In this type of allergy, the immune complex reactions initiate an inflammatory response due to the combination of the excess drug- protein complex with the IgG in circulation. The complex thus formed is deposited in the tissues and causes activation of the complement and damage of capillary endothelium. This type of reaction is manifested mostly as fever, arthritis, and/or enlarged lymph nodes. Penicillins, sulphonamides and streptomycin may elicit type III allergic reactions.

Type IV

This is the cell-mediated or delayed hypersensitivity reactions in which the T - lymphocytes are sensitized by a hapten to form protein-antigenic complex such that when the lymphocytes come into contact with the antigen, an inflammatory response ensues. Type IV reactions are exemplified by contact dermatitis caused by local anaesthetic areas, antihistamine areas, topical antibiotics and antifungal drugs.

Type V

These are pseudo allergic reactions, that resemble allergic reactions clinically, but for which no immunological basis can be found, e.g. asthma and skin rashes caused by aspirin. Admin-

istration of ampicillin or amoxicillin causes a skin rash which resembles the one caused by penicillin hypersensitivity. The ampicillin-caused rash can be distinguished from penicillin hypersensitivity on the basis of two features; it has a later onset, typically ten to fourteen days, compared to penicillin sensitivity which comes between seven to ten days. Furthermore, the sensitivity does not recur following re-exposure to ampicillin and is not as serious as the one caused by penicillin.

14.20. Clinical evaluation of adverse drug reactions

The two basic approaches for clinical evaluation of adverse drug reactions include the cohort studies (or follow-up studies) of patients taking the drug and the case control studies which record the incidences of adverse drug effects retrospectively.

Cohort or prospective studies

In cohort studies, drugs are identified and incidences of adverse effects recorded. The weaknesses of these studies include; the relatively small number of patients likely to be recruited, and lack of suitable control groups to assess the background incidence of any apparent adverse reaction noted.

Case control or retrospective studies

The approach here is to start with the incidence of adverse reaction(s) and then look for the drug and the individuals with symptoms which could be due to an adverse drug reaction. These individuals are screened to see if they had taken the drug. The prevalence of drug taking in the group is then compared with the prevalence in a reference population which did not take the drug. This approach is excellent for validation and assessment of adverse drug effects, but it may not detect new adverse effects. Furthermore, it requires a very large number of patients and is very expensive to undertake hence difficult to justify and organize for every new product.

14.21. Worked examples

Problem 5

Drug clearance must always be adjusted for alterations of renal function using the formula: $rf_{pt} = 1 - fe_{nl}(1 - rfx_{pt})$, where fe_{nl} = fraction of the drug excreted unchanged in normal individuals, rf_{pt} = adjustment factor for total clearance in patient, rfx_{pt} = patients' clearance as a fraction of normal clearance and Cl_{nl} = normal clearance.

Given that an asthmatic patient has a creatinine clearance of $40\text{mlmin}^{-1} 70\text{kg}^{-1}$ and that the fraction of terbutaline excreted unchanged, $fe_{nl} = 0.56$, the normal clearance, $Cl_{nl} = 3.4\text{ ml/min/kg}$, calculate the clearance of the drug in the patient.

Solution

The patient has depressed renal function: $rfx_{pt} = (40\text{ ml/min}) / (120\text{ ml/min}) = 0.33$

$$rf_{pt} = 1 - fe_{nl}(1 - rfx_{pt})$$

inline formula $rf_{pt} = 1 - 0.56(1 - 0.33) = 0.62$

$$Cl_{pt} = Cl_{nl} \cdot rf_{pt}$$

inline formula $= 3.4 \text{ ml} \cdot \text{min}^{-1} \text{ kg}^{-1} \times 0.62$
 $= 2.1 \text{ ml} \cdot \text{min}^{-1} \text{ kg}^{-1}$

Problem 6

Given the following characteristics of drug A; $t_{1/2} = 8\text{h}$, given at a dosage of 450mg every 12h, has $V_{ss} = 0.5 \text{ L/kg}$, effective concentration is 12mg/L and that

$$C_{ss \text{ min}} = \frac{F \times \text{dose} / V_{ss} (\exp^{-kt})}{1 - \exp^{-KT}}$$

$$C_{ss \text{ max}} = \frac{F \times \text{dose} / V_{ss}}{1 - \exp^{-KT}}$$

Determine the $C_{ss \text{ min}}$ and $C_{ss \text{ max}}$ for a 60 kg patient if $F = 1$ and $\exp^{-KT} = 0.35$. Explain how changing of the dosage interval to 6 hours would affect $C_{ss \text{ min}}$.

Solution

The term \exp^{-KT} is the fraction of the last dose that remains in the body at the end of a dosing interval and is equal to 0.35 and $C_{ss \text{ min}} = C_{ss \text{ max}} \cdot \exp^{-kt}$. Therefore,

$$C_{ss \text{ min}} = \frac{450/30 \times 0.35}{1 - 0.35} = 15/0.65 \times 0.35 \sim 8.0 \text{ mg/L} \text{ while } C_{ss \text{ max}} = 15/0.65 = 23 \text{ mg/L}$$

The predicted minimum of 8.0mg/L is below the effective concentration to achieve efficacy. Therefore the dosing interval should be reduced. A reduction of the interval to, say, six hours, increases denominator and therefore causes an increase of $C_{ss \text{ min}}$. Since $t_{1/2} = 0.693/K$, $K = 0.086$ i.e. $1 - e^{-KT} = 1 - 2.71^{-0.086 \times 6} = 1 - 0.596 = 0.4$.

The new $C_{ss \text{ min}}$ becomes; $= 450/30 \times 0.4 = 15 \text{ mg/L}$ which is within the required therapeutic concentration.

Review exercise

1. Write an essay on statistical considerations that guide clinical evaluation of new drug agents
2. Write an essay on bioprospecting for new antimicrobial agents.
3. Drug clearance must always be adjusted for alterations of renal function using the formula: $rf_{pt} = 1 - f_{enl}(1 - rf_{pt})$, explain what each term in the above equation represents. Consequently, calculate the clearance of acetaminophen (panadol) in a 70 kg patient with depressed renal function given the following; normal clearance = $350 \text{ ml min}^{-1} 70 \text{ kg}$; $f_{enl} = 0.56$. The patient creatinine clearance = $80 \text{ ml min}^{-1} 70 \text{ kg}$. Normal creatinine clearance = $120 \text{ ml min}^{-1} 70 \text{ kg}$. What effect would the impaired renal function have on dosing interval?

4. Given that $f_{e_{nl}}$ for drug X = 0.42, and the normal creatinine clearance is 120 ml/min/70 kg, calculate the clearance rate of the drug by a patient with creatinine clearance of 75 ml/min/70 kg.
5. Write an essay on adverse drug reactions associated with the use of macrolide antimicrobial agents.

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