Unit 5

MECHANISM OF TOXICITY

Structure

5.1 INTRODUCTION

To show systemic toxicity, toxicants must be absorbed in the blood and delivered (or distributed) to the target organ. Once toxicants have entered the body, they are enzymatically modified (metabolized) and excreted. The efficiency of absorption, distribution, metabolism and excretion (ADME) affects attainment of toxic concentration and duration of stay of toxicants in the body. Each of these processes is affected by several factors including physicochemical properties of toxicants, anatomical features of the body, genetic and physiological factors, and environmental factors. In this unit, we will discuss the process, parameters to measure the efficiency, and factors affecting ADME.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ understand the process of ADME orhow toxicants enter the body, move to various organs, areenzymatically modified and removed from the body,
- ❖ know factors affecting the ADME,
- ❖ know role of organs such as liver, gastrointestinal tract, lungs and skin in disposal and activation or toxicants and,
- ❖ learn basics of how to quantify or measure the efficiency of ADME processes.

5.2 INTRODUCTION TO ADME—ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF TOXICANTS

We have already discussed in the previous units that systemic toxicity happens when:

1. a toxicant is 'absorbed' from the site-of-exposure (such portion of skin, gastrointestinal tract, lungs, etc.) and reaches systemic circulation,
2. followed by its 'distribution' via blood to the site-of-toxicity or target organ,
3. its interaction with the target molecule in the target organ resulting in alteration of the function of the former,
4. which in turn causes alteration in function of cell/irreparable injury to cells, followed by abnormality in the function of organ and manifestation of toxicity,

The above process usually happens in a step wise manner. However, while toxicants are being absorbed and distributed, these simultaneously undergo 'metabolism' (also known as biotransformation) and 'excretion'. The extent and rate of Absorption, Distribution, Metabolism and Excretion (ADME) thus determines the 'concentration' and 'duration of stay' of toxicants in the body (Unit 3). The entire process can be diagrammatically summarized as given in Fig. 5.1.

The quantitative study (i.e. rate and extent) of ADME of toxicants is known as 'toxicokinetics'. In this section, we will study the qualitative aspects i.e. mechanism and factors affecting absorption, distribution, metabolism and excretion.

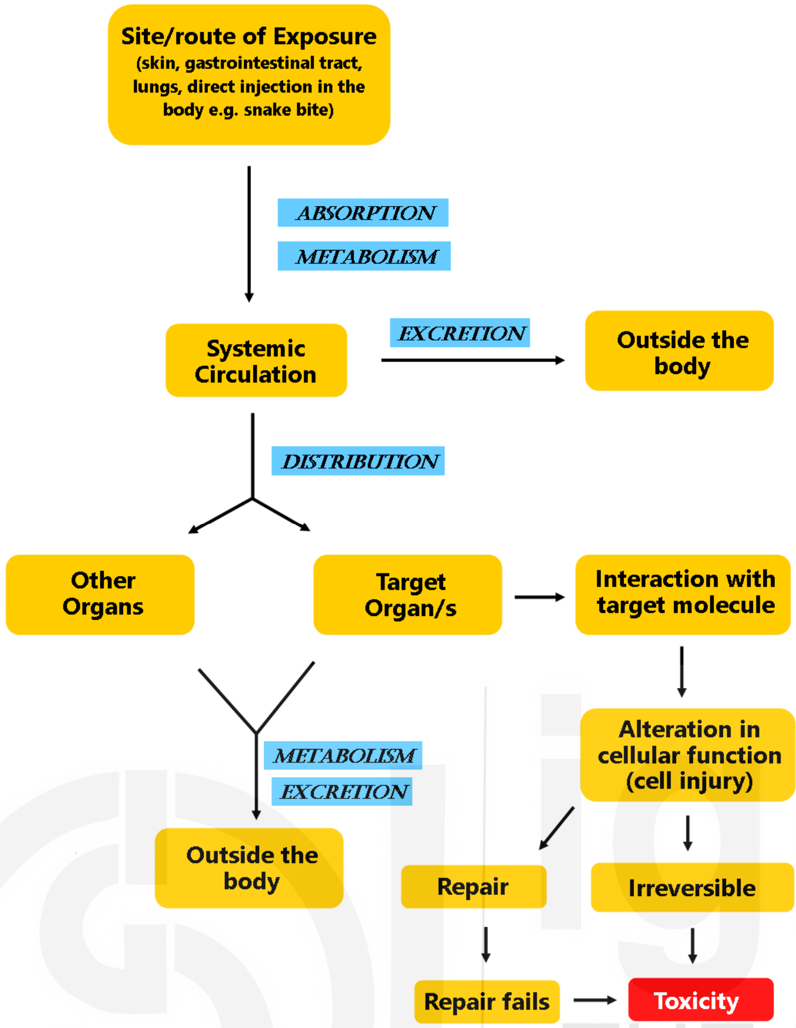

Fig. 5.1: Overview of the events leading to toxicity.

5.2.1 Absorption

Absorption is the process by which toxicants cross various tissues (epithelial layer, lamina, sub-mucosa, dermis, etc.) at the site-of-exposure and enter systemic circulation.To absorb and reach bloodstream from the site-of-exposure, toxicants have to cross cell membranes, move through intercellular space in the tissues, and again cross a cell membrane to enter another tissue or blood vessels. Poor absorption of toxicants may not initiate a toxic response (see Unit 3).

The extent and rate of absorption depends on the physicochemical properties of toxicants (lipid solubility, size, etc.) and attributes of the site where absorption takes place: i.e. surface area, properties of epithelium (presence of transporters, thickness of epithelium, etc.) and blood supply in the underlying tissue. In general, absorption readily takes place if the toxicant is lipid-soluble, and the site of absorption has a large surface area and good supply of blood.

The efficiency of absorption is expressed by the term ‘bioavailability’ which is defined as ‘fraction of administered chemical reaching the systemic circulation’. We will discuss more about bioavailability at the end of this section.

The common routes through which humans are exposed to most of the toxicants are oral (ingestion), lungs (inhalation), and skin surface. Below, we will discuss absorption of toxicants via these routes and factors affecting rate and extent of absorption.

Presystemic Elimination

Presystemic elimination is the removal or inactivation of the absorbed toxicant before it reaches systemic circulation for distribution to the target site/s. Two processes are mainly responsible for presystemic elimination, viz. i) inactivation of absorbed toxicant due to biotransformation in liver, enterocytes (epithelial cells of gastrointestinal tract), and lungs; 2) excretion oftoxicants without or after biotransformation into bile by liver. The overall effect of presystemic elimination is to reduce the total amount of toxicant absorbed from the site-of-exposure before it reaches systemic circulation (thus decreasing the bioavailability). Toxicants absorbed in gastrointestinal tract undergo significant presystemic elimination. We will see more about biotransformation in subsection 5.2.3.

Absorption via oral route: Toxicants entering via oral route are absorbed in gastrointestinal tract. Though some amounts of toxicants can be absorbed in the buccal cavity, most of it is absorbed in the stomach and intestine. After being absorbed in gastrointestinal tract, toxicants are carried first to the liver (Fig. 5.2). Here, toxicants may become substrates for 'xenobiotic metabolizing enzymes' and undergo enzymatic reactions resulting in modification of their chemical structure–a process known as 'biotransformation' or 'xenobiotics metabolism' (see below). Biotransformation may inactivate a fraction of absorbed toxicants and promote their excretion before they reach systemic circulation–'Presystemic elimination'.

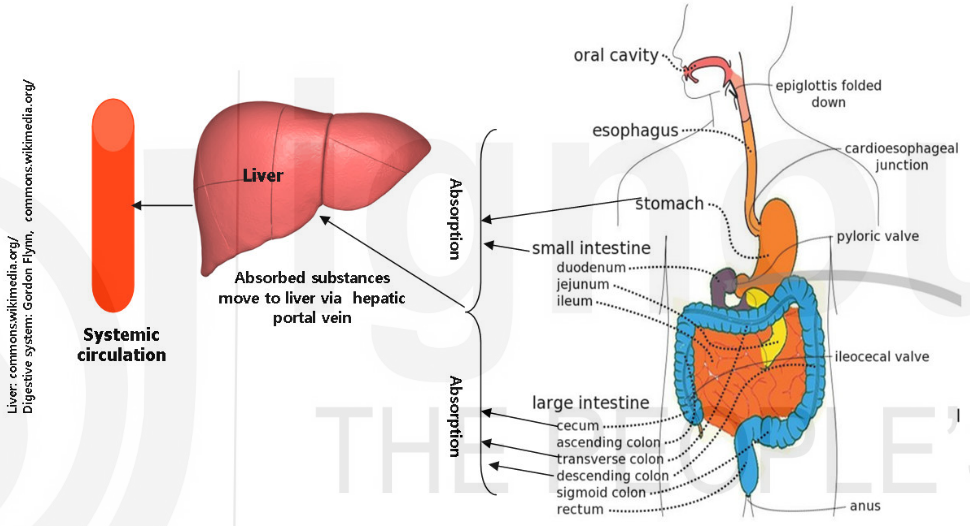

Fig. 5.2: Absorption and movement of toxicants exposed via oral route.

Lipid-soluble toxicants can cross cell membrane by *simple diffusion,* and thus, are absorbed efficiently. Toxicants (such as those with weak acidic groups) that remain predominantly in uncharged form at acidic pH (thus more lipid-soluble than the charged form) are absorbed well in the stomach. Conversely, organic bases are predominantly in charged form in stomach and are poorly absorbed here. However, a more basic environment of intestine causes molecules of organic bases to lose charge (thus more lipid-soluble) and are absorbed efficiently. For example, salicylic acid (pKa=2.19) remains uncharged in low pH conditions of stomach (pH=2) and is well absorbed, whereas aniline (pKa 4.6) is uncharged in more basic pH of intestine (pH=6–7.4) and is absorbed well here. From above discussion, we learnt how pH of the surroundings can affect absorption of toxicants. Small water-soluble toxicants are not able to cross cell membrane by simple diffusion. They get access to the body by *facilitated diffusion* and *active transport* using membrane transporters, or diffuse via *aqueous pores* between two adjacent cells. Large particulates are absorbed via the process of *pinocytosis* (Fig. 5.3).

Absorption of toxicants via gastrointestinal tract is affected by the presence of food, certain components of food and motility of the intestine. Let us see few examples of these: i) citrate is widely used as a flavouring agent. It causes widening the paracellular space by disrupting the tight junctions between adjacent cells of intestinal epithelium. Citrate chelates calcium ions involved in stabilization of tight junctions. Citrate therefore, increases absorption of substances including aluminium, lead, etc, ii) inorganic mercury may react with the thiol group (–SH) containing substances in food (such as cysteine) and the resultant complex gets absorbed by using amino acid transporters, iii) laxatives decrease the absorption of toxicants by increase the motility of intestine (condition similar to diarrhoea), thus reducing the total time for which toxicants stay in the intestine.

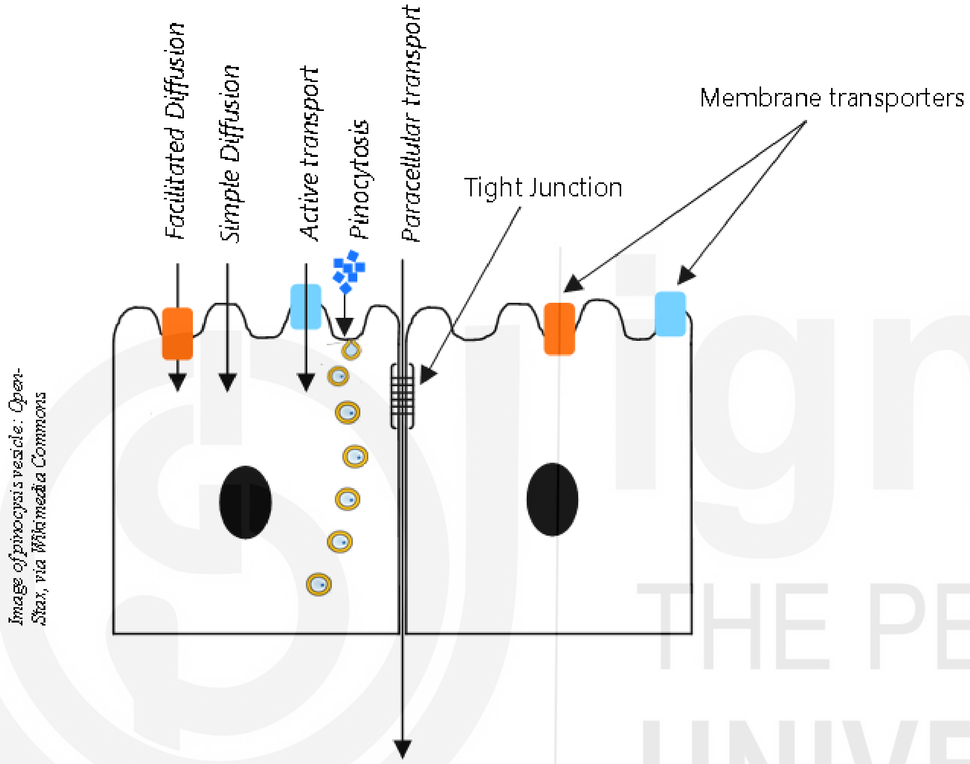

Fig. 5.3: Various ways in which toxicants can cross cell membrane.

Absorption via lungs: Inhaled toxicants (gases, vapours of volatile liquids, aerosols and particles) are mainly absorbed in the lungs. The principles of absorption of gases and vapours and, aerosols and that of particles are different, so we will discuss them separately.

Inhaled molecules of gases and vapours fill the alveolar space in the lungs from where these diffuse across the thin alveolar epithelium and enter the blood. Toxicants dissolved in blood are then quickly carried to other parts of the body. The inspired gas keeps dissolving in the blood from alveolar space till an equilibrium is established at which the ratio of concentration of gas in blood and alveolar sac is constant. This ratio is called ‘blood-to-gas’ partition coefficient and indicates solubility of gas in blood with respect to alveolar air. The greater the blood-to-gas partition coefficient, greater is the solubility and faster it will dissolve in blood with each inhalation. However, a more soluble gas slowly reaches equilibrium and more cycles of inhalations (or time) are required to equilibrate with the blood. Anaesthetic gases with high blood-to-gas partition coefficient dissolve in higher concentration in blood, however, have slower rate of induction of anaesthesia.

The principal governing factors for the absorption of aerosol and particles are the size of aerosols and water solubility. Coarser particles (5 micron or larger) are mainly deposited in the nasopharyngeal region. Particles soluble in mucus lining of the nasal passage may get absorbed, or removed by sneezing or nasal wiping. Fine particles (about 2.5 micron) reach deeper in the respirator tree, i.e. tracheobronchiolar region. These may be displaced from here together with mucus towards mouth, and swallowed or spited out. In the former situation, the swallowed toxicant may be absorbed in gastrointestinal tract. Ultrafine particles (1micron or less) can penetrate further deeper into the alveolar sac. These may get absorbed in blood or reach lymphatic system after being phagocytosed by alveolar macrophages.

Absorption via skin: Our skin comes in contact with several chemicals including potent toxicants (e.g. farmers during formulation and application of pesticides). Skin acts as a barrier and prevents or reduces the amount of several toxicants from reaching the blood. Skin, however, is not an absolute barrier and many toxicants can penetrate through it to access blood.

Skin is comprised of three layers; the outer ‘epidermis’, below which lies ‘dermis’, and the innermost fatty layer ‘hypodermis’. Epidermis is a multilayered structure, and the outermost layer of epidermis the ‘stratum corneum’ or horny layer is composed of densely packed flat cells lacking nuclei, and embedded in a lipid rich extracellular matrix. Stratum corneum represents an important barrier to absorption of toxicants. The second layer– dermis, is a connective tissue layer rich in blood supply. Dermis is interrupted by several tubular structures i.e. sweat glands, oil glands and hair follicles (collectively called ‘dermal appendages’). Dermal appendages open at the surface of skin by traversing stratum corneum (Fig. 5.4). Significant absorption of toxicants may take place through dermal appendages.

To get absorbed in blood, a toxicant must reach dermis and move into the blood vessels.

In general: i) stratum corneum poses a barrier to large water-soluble chemicals,however, small fat-soluble toxicants can cross this layer and reach dermis ii) water-soluble chemicals can penetrate skin and reach dermis via dermal appendages.

Several factors can affect absorption of toxicants via skin, such as– size of toxicant molecules, temperature of skin, solvent in which toxicant is dissolved, and moisture level of stratum corneum. Chemicals with molecular weight of >500 Daltons are poorly absorbed via skin, solvents (such as acetone) and excessive application of soaps can disrupt the integrity of stratum corneum, making skin more permeable for toxicants. Hydration of skin such as during sweating increases permeability of skin for certain toxicants. High temperature increases blood flow in dermis thereby removing absorbed toxicants at a faster rate; and maintains a concentration gradient for further absorption of more toxicant. Sweating and high temperature together can cause significant absorption of toxicants in people handling hazardous substances at their workplace (factory workers, farmers) in tropical climatic conditions.

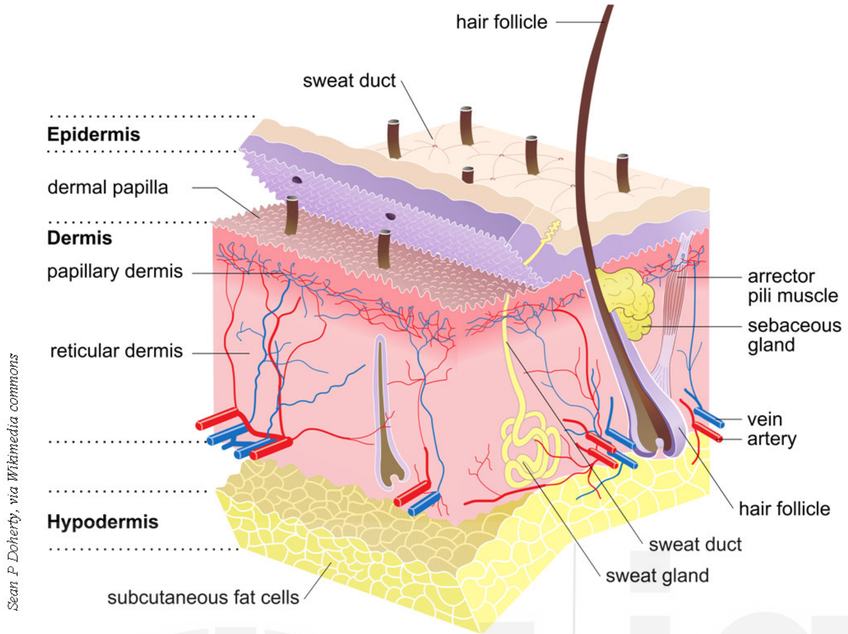

Fig. 5.4: Illustration of skin showing anatomical features. (Source: Image credits Sean P Doherty, CC BY-SA 4.0 httpscreativecommons.orglicensesby-sa4.0, via Wikimedia Commons)

5.2.2 Distribution

After a chemical enters systemic circulation, it travels to various organs of the body including its target organ via blood–a process known as distribution. After reaching the target organ, toxicants move out of the blood capillaries and enter various cells of the organ. While inside an organ, toxicants may encounter their target molecules (if found in that organ) and interact with the latter and show toxic response. Students may note that toxicants are not homogeneously distributed in all parts of the body i.e. their concentrations are not same throughout the body after distribution. There are several factors responsible for this heterogeneous distribution, of which we will discuss a few major factors here:

To enter various organs and cells of each organ, toxicants must cross cell membranes of capillary endothelial cells and those of cells of the organs. Recall, in the absorption section we learnt that–'whether a chemical will be able to cross cell membrane depends on its physicochemical properties and properties of cell membrane'. Thus, lipophilic (fat-soluble) toxicants are widely distributed in the body, whereas, lipophobic (water-soluble) toxicants have restricted distribution. For example, the blood-brain barrier prevents many water-soluble chemicals from entering brain, whereas fat-soluble chemicals can easily enter brain by simple diffusion.

In addition to the above-mentioned properties, toxicants that are extensively bound tothe plasma proteins (such as albumin) are mainly restricted to blood vascular system, because it is only the free or unbound form that can move out of the blood vesselsby crossing capillary endothelial cells. Further, certain toxicants tend to accumulate in a particular tissue of the body, and these tissues are known as ‘storage depot’. For example, fat-soluble toxicants such as DDT, TCDD, etc., accumulate in fatty tissues (adipose tissue), cadmium is stored in liver in conjugation with a protein named ‘metallothionein’, and most of the lead and strontium to which humans are exposed are found in the matrix of bones in conjugation with hydroxyapatite crystals. Toxicants accumulated in the storage depots may remain there for several years. The concentration of such toxicants is usually too low in the blood to show a toxic response. However, during periods of quick mobilization of toxicants from the storage depot, a toxic concentration is attained in the blood that may result in toxic response. For example, DDT stored in adipose tissue is mobilized into blood at a faster rate during starvation and weight loss. This causes increase in exposure of internal organs to high concentrations of DDT and may cause toxicity. Let us see another example: about 80–90% of the lead we absorb is stored in bones. Depending on the extent of exposure, over the years, a high amount of lead can get accumulated in the bones. In postmenopausal women, lead can be mobilized from the bones due to age related osteoporosis, with its resultant increase in blood leadingtotoxic levels.

The extent of distribution of toxicants (or xenobiotics) in the body is measured by ‘Volume of Distribution’ (V). It is defined as ‘virtual volume of the body in which toxicants appear to distribute’.

$$\text{Volume of distribution (V)} \quad \frac{\text{Amount of toxicant administered (mg)}}{\text{Concentration of toxicant in blood (mg/L)}}$$

Suppose,

Amount of toxicant administered= 200 mg; concentration of toxicant in blood= 4 mg/L

V= 200/4= **50 L**

So, a toxicant with lower value of ‘V’ is expected to remain in the blood vascular system or have limited distribution, whereas a toxicant with higher ‘V’ (such as lipid-soluble substances) is expected to leave blood vascular system and enter various organs of the body.

5.2.3 Metabolism

Metabolism of xenobiotics is also known as biotransformation. It is aprocess during which certain enzymes of our body (called xenobiotics metabolizing enzymes) modify the chemical structure of parent chemicals (original form in which a chemical was exposed). There are several fates of a chemical after it undergoes biotransformation: i) it may be converted into an inactive metabolite (detoxification or bio-inactivation), ii) an otherwise non-toxic chemical is converted into a potent toxicant (bio-activation or toxication), iii) a lesser toxic chemical is converted to more a potent toxic metabolite (bio-activation).

Though xenobiotics metabolizing enzymes are found in many tissues of many organs in the body, their highest concentration is found in liver and enterocytes (epithelial cells of gastrointestinal tract).Thus, these two organs are usually responsible for the biotransformation of most of xenobiotics to which humans and animals are exposed.

Humans (and animals) have limited number of xenobiotic metabolizing enzymes, and these havebroad and sometimes overlapping substrate specificities. For example, humans have 57 cytochrome P450 monooxygenase (CYP 450) enzymes and one of the isoforms- CYP 3A4 is known to metabolize more than 30% of the drugs. In many cases, a toxicant can be metabolized by more than one enzyme, for example, ethanol can be metabolized by three enzymes, viz. CYP450, alcohol dehydrogenase and catalase.

Biotransformation reactions can be grouped into two categories:

Phase I reactions: includes oxidation, reduction, and hydrolysis reactions,

Phase II reactions: conjugation reactions: attachment of hydrophilic endogenous molecules (glucuronic acid, glycine, glutathione and sulphate) to phase I metabolites or parent toxicants.

The significance of biotransformation reactions is that, they convert fat-soluble toxic substances into their more water-soluble metabolites that can be quickly excreted from the body (via urine, bile, sweat, etc.). This reduces the total duration for which toxic compounds stay in the body. For example, 'benzene' entering the body is converted to 'phenol'in phase I reaction. Phenol is then conjugated with sulphate in phase II reactions which not only detoxifies it, but also makes it more water soluble and easily excretable.We will discuss main factors which affect the metabolism of xenobiotics in section 5.4. Fig. 5.5, depicts the events contributing to elimination of toxicants from the body.

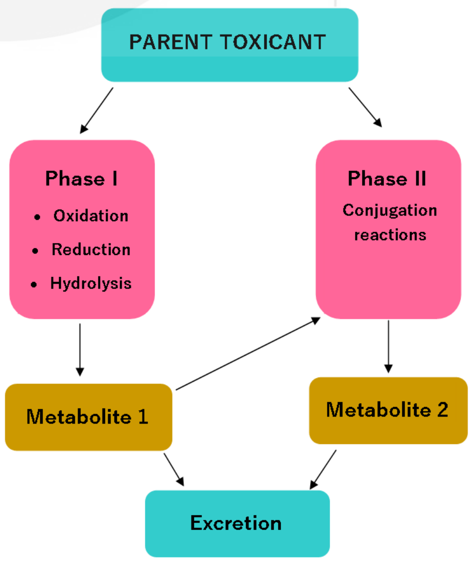

Fig. 5.5: Schematic of elimination of toxicants form the body.

5.2.4 Excretion

Excretion is removal of a substance (or toxicants) from blood to the external environment. The major routes through which various substances are excreted include urine, faeces, exhalation via lungs. Toxicants may also be removed from the body via minor routes including sweat, saliva and milk.

Urine: Excretion of toxicant via urine is the most important route; usually water-soluble substances are efficiently excreted in urine. Lipid-soluble substance sare reabsorbed efficiently from the kidney tubular fluid, thus, their residence time in blood is more. Since, pH of the medium affects the ionization of weak acid and bases (and thus the lipid-solubility, see above), the pH of urine can affect the rate of excretion of toxicants. This principle is used for speedy removal of salicylate (a weakly acidic chemical) from the body in cases of poisoning. By administering sodium bicarbonate, the pH of the urine is raised, and a high pH converts more salicylate molecules into ionized form (more water-soluble), and are removed by the kidneys at a faster rate.

Faeces: itis the second important route of excretion. Toxicants excreted in faeces originate from: 1) unabsorbed toxicants in gastrointestinal tract, 2) toxicants (or metabolites) secreted from blood into intestinal lumen and, 3) toxicants entering gastrointestinal tract via bile. The presence of toxicants in bile is due to a process whereby certain membrane transporters move toxicants (or conjugate formed in phase II reactions) from hepatocyte into bile canaliculi and ducts. Sometimes conjugates of toxicants carried via bile inthe intestinal lumenare deconjugated by intestinal or microbial enzymes (recall gut microflora), and the resulting parent toxicants are reabsorbed; and again, brought to the liver. This cycling of toxicants from the liver to intestine and back is called ‘entero-hepatic cycling’. Repeated entero-hepatic cycling increases the residence time of toxicants in the body and thus requires interruption of the cycle. For example, victims/patients of poisoning are given activated charcoal via oral route, conjugates of toxicant entering intestine via bile are adsorbedto the charcoal and thus, are prevented from reabsorption.

Lungs: Gases and volatile liquids dissolved in blood are excreted by exhalation via lungs. The toxicants diffuse from blood to alveolar sac and are then exhaled. Substances with higher solubility in the blood (chloroform) are removed at slower rate than those with relatively low solubility (ethylene).

Milk: Lipid soluble toxicants get dissolved in the fat portion of milk (during synthesis in mammary gland) and are excrete from the body. Several toxicants including DDT, PCBs, aldrin, have been found in human milk. The secretion of toxicants in milk is of concern because it may pass from the mother to suckling, and to the general population via milk and milk products prepared from farm animals.

5.2.5 Bioavailability

We defined bioavailability above as–fraction (or percentage) of administered (or exposed) toxicant that reaches the systemic circulation intact or in original form. Bioavailability is a measure of the amount of a chemical that is absorbed from the site-of-exposure and has escaped presystemic elimination. Chemicals directly introduced into veins are considered to have 100% bioavailability. Chemicals administered (or exposed) via other routes have lower bioavailability due to incomplete absorption and, presystemic elimination. Therefore, only a fraction of administered amount reaches systemic circulation, and is available for distribution to the target site. Toxicants exposed via oral route usually undergo considerable presystemic elimination in enterocytes and liver, thus, have significantly low bioavailability. It is therefore possible that oral LD50 value of a toxicant may be higher than the LD50 value measured via other routes of exposure. For example, LD50 value for carbaryl (an insecticide) in rats via oral, intraperitoneal and intravenous routes are 230 mg/kg, 64 mg/kg, and 18 mg/kg respectively.

Within a population, there may exist a variation in bioavailability among individuals (exposedto a given chemical via same route). This is due to biological variations (genetic differences in the xenobiotics metabolizing enzymes, transporters, etc.), concurrent exposure to another chemical, age, gender, etc. (see below)

To estimate Bioavailability (F), the curve between 'plasma toxicant concentration' verses 'time' is plotted following exposure of animals via oral (or other test routes), and intravenous route. From these curves, the area under curve (AUC) is determined and 'F' can be estimated by the following formula:

$$\text{Bioavailability (F)} = \frac{\text{AUC via test route}}{\text{AUC intravenous route}} \times 100$$

5.2.6 Clearance

Clearance (CL) is a parameter used to quantify elimination of xenobiotics. It is defined as 'volume of plasma from which a substance is completely removed per unit time'. The unit of clearance is 'millilitres/minute' or 'litres/hour'. Clearance is thus indicative of the rate at which a substance is removed from the body–high clearance value means rapid removal of a substance from the blood or body.Since most substances are excreted or removed by the kidneys, the term 'renal clearance' (CL_R) is used frequently instead. Further, substances may be excreted via several organs viz. kidneys, bile, lungs, etc. In these cases, clearance is the sum of clearance via each of these organs thus, the 'total body clearance' or 'overall clearance' $=CL_R+CL_B+CL_L$.

$$\text{Total Clearance } (CL_{TOT}) = \frac{\text{Rate of elimination of xenobiotics}}{\text{Plasma concentration of toxicant}}$$

5.2.7 Elimination Half-life

Elimination half-life ($t_{1/2}$) is the time taken to reduce the concentration of axenobiotic in the blood by half. Thus, after one half-life, the concentration of a xenobiotic will be half of the initial concentration. Elimination half-life indicates the rapidity with which a substance is removed from the blood or the duration for which it would stay in the blood. Elimination '$t_{1/2}$' depends on clearance (CL) and volume of distribution (V):

$$t_{1/2} = \frac{0.693 \text{ X } V}{CL}$$

It can be deduced from above formula that large 'V' and low 'CL' increase '$t_{1/2}$'. Thus, lipid-soluble chemicals which are widely distributed stay for longer duration in the body (larger $t_{1/2}$ value).

5.2.8 Total body Burden

Total body burden refers to the amount of xenobiotics in the body at a given period of time.

Throughout our life, we are exposed to millions of xenobiotics, some of which stay in the body only for a short period of time (arsenic $t_{1/2=}$10 hours), whereas others stay for a longer duration or accumulate in the body (DDT $t_{1/2=}$ 3-6 years). However, a continuous exposure to chemicals with shorter $t_{1/2}$ can cause their persistent presence in the body. For example, continuous exposure to arsenic by regularly consuming contaminated food and water results in its persistent presence in the body. According to an estimate, humans may be carrying over 200 different xenobiotics at a time in the body. The presence of a toxic substance in the body does not necessarily indicate a state of toxicity, for which the chemical must be present above a threshold concentration levelin the body. However, measurement of total body burden gives information about the past and current exposure to chemicals, and a more realistic exposure assessment of chemicals (concentration to which organs are actually exposed) than monitoring concentration of chemicals in the environment. To estimate total body burden, samples of blood, urine, milk, hair, etc. are collected from a population and quantitatively analyzed–a process known as bio-monitoring.Such monitoring may indicate the overall state of toxic substances in environment and public health.

SAQ 1

a) State whether the following statements are true or false:

i) Toxicants absorbed via oral route usually have a high bioavailability. [True/False]

ii) Stratum corneum of skin is highly permeable to hydrophilic chemicals. [True/False]

iii) Liver is the main organ for the biotransformation of xenobiotics. [True/False]

iv) Toxicants accumulated in storage depots may show toxicity sometimes at the later stage of life. [True/False]

v) Entero-hepatic cycling of toxicants increases the elimination half life. True/False]

vi) The extent and rate of ADME is the same in all individuals of a species. [True/False]

b) Fill in the blanks with appropriate words:

i) The unit of clearance (CL) is......................

ii) Hydrophilic chemicals in intestine are absorbed by transport mechanisms such as ....

iii) The amount of xenobiotics in the body at a given point of time is called...........

5.3 ROLE OF LIVER, LUNGS, GASTROINTESTINAL TRACT AND SKIN IN ACTIVATION AND DETOXIFICATION OF TOXICANTS

We have seen above that the main routes through which toxicants enter our body are gastrointestinal tract (ingestion), lungs (inhales) and skin (contact with skin). Toxicants entering via these routes undergo biotransformation before they advance to systemic circulation. Liver and enterocytes of intestine, limit the amount of orally ingested toxicants by detoxification before these toxicants reach systemic circulation. Similarly, lung sand skin though less important than liver in xenobiotics metabolism, also detoxify certain toxicants entering via inhalation and via skin surface. Significant detoxification can reduce bioavailability of toxicants and prevent occurrence of a toxic response. In this regard, biotransformation reactions protect the body from toxic hazards. However, in many cases, biotransformation reactions convert an inactive parent toxicant into a potent toxicant– a process known as 'toxication' or bioactivation. For example, inhaled hexane is metabolized in liver intoan active neurotoxic metabolite by CYP450. In another example, biotransformation of ingested paracetamol produces certain hepatotoxic metabolites in phase I reactions, these, however, are quickly detoxified by phase II reaction and no toxicity is observed. Thus, it is the balance between the activation and detoxification process that actually determines occurrence of toxicity.

SAQ 2

State whether the following statements are true or false:

i) Sometimes a chemical may be activated to a potent toxicant in liver. [True/False]

ii) Biotransformation reactions protect body from toxic insult. [True/False]

5.4 FACTORS AFFECTING METABOLISM OF XENOBIOTICS

There are variations in metabolism of xenobiotics amongst individuals of different species (interspecific) and individuals of same species (intraspecific). Pesticides selectively harming target species and variation in response to drugs within human population can be partly explained by the differences in xenobiotics metabolism. There are several factors responsible for the variation in xenobiotics metabolism; we will discuss a few key factors in the following section:

5.4.1 Species

Differences in xenobiotics metabolism amongst various animal species can be due to: absence of the entire pathway of metabolism of xenobiotics in some species; presence of a low efficiency pathway in some species as compared to others and; presence of alternate pathways for metabolism of a xenobiotic in some species. These differences cause: some species to form toxic metabolites (or at a faster rate) of xenobiotics while others do not;some species are unable to detoxify xenobiotics at faster rate or; metabolize toxicants through multiple pathways thereby preventing accumulation of activated metabolite produced in one of the alternate pathways. Let us see few examples to explain the above situations:

1. Cats have lesser ability to conjugate xenobiotics with glucuronic acid in phase II biotransformation reactions, required for its rapid clearance from the blood. Thus, in cats, half-life of some xenobiotics is prolonged, andexplains the susceptibility of cats to the toxic effects of paracetamol. Similarly, the enzyme N-acetyltransferase responsiblefor acetylation of xenobioticsin phase II reactionsis absent in dogs, due to which the half-life of xenobiotics (e.g. hydralazines) is increased and results in toxicity.

2. Malathion, an insecticide is converted to malaoxon (the active form) in both humans and insects. However, humans rapidly detoxify malaoxon due to greater carboxylesterase activitythan insects. Insects thus accumulate malaoxon in their body, which explains species selective toxicity of malathion, towards insects.

3. Rats metabolize ‘coumarins’ into a toxic intermediate by epoxidation reaction.Humans however, use a different enzyme to metabolize coumarins and produce a metabolite which is non-toxic.

4. Tamoxifen, is used to treat breast cancer. It is carcinogenic in rats but not in humans. This difference is due to the fact thatthe rate of formation of toxic metabolite of the drug is slower, whereas detoxification pathway is more efficient in humans than rats. Thus, rats can accumulate several times higher amounts of the genotoxic metabolite in the body than humans.

From the above discussion, it can be deduced that significant differences in the ability to metabolize xenobiotics exists among various species; and is one of the important reasons for species selective toxicity. It also explains why certain pesticides do not harm humans or are toxic if taken at higher amounts.

5.4.2 Genetic Factors

Polymorphism in drug metabolizing enzymes causes inter-individual variations in the xenobiotics metabolism in a population. Several alleles of one or more xenobiotics metabolizing enzymes exist in a population, which produce enzymes that catalyze reactions at different rates. Thus, it is possible thatsome individuals in a population harboura particular allele which makes them metabolize a xenobiotic faster than other individuals. Such individuals are denoted as ‘rapid metabolizers’, and those metabolizing at slower rate as ‘poor metabolizers’. Similarly, individuals with intermediate metabolizing capacity are called ‘intermediate metabolizers’. Let us take the example of ‘succinylcholine’, a drug used to cause muscle relaxation required duringthe intubation of patients. This drug is rapidly metabolized by ‘pseudocholinesterase’ enzyme, which leads to the termination of drug action. In individuals with the normal allele, muscle relaxation lasts for about 8-15 minutes. However, individuals with abnormal allele show prolonged muscle relaxation which lasts for a few hours accompanied by breathlessness. Genetic polymorphism in several isoforms of CYP450 makes certain individuals of the population more susceptible totoxicants. For example, the isoform CYP2A6 metabolizes ‘nicotine’ and activates tobacco-specific mutagens. Individuals with abnormal allele poorly metabolize and activate tobacco-specific mutagens, thus, reducing the possibility of lung cancer.

From above discussion students may have understood that how genetic differences in xenobiotics metabolizing enzymes in a population can cause variation inxenobiotics metabolism and thus outcome of the toxic response.

5.4.3 Physiological Factors

Sex: There are differences in the xenobiotics metabolism between the two sexes of the same species. This is however not as pronounced in humans and other species as in the laboratory rats, in which male can metabolize several compounds at higher rates than the females. This is due to the dissimilarity in the expression of metabolizing enzymes between the two sexes. Thus, the activation or detoxification of xenobiotics between the two sexes may also differ. Lets us explain the above by using a few examples:

- Men have higher activity of CYP1A2 than women, due to which men can metabolize caffeine at a faster rate. Also, females require lower dose of hypnotic zolpidem (also metabolized by CYP1A2) due to slower rate of metabolism than males.

- After consumption of alcohol, females show higher alcohol levels in blood than males. This is because women have lower activity of gastric alcohol dehydrogenase, an enzyme responsible for metabolizing or degrading alcohol before it reaches systemic circulation. Thismakes women more sensitive to alcoholtoxicity than men.

Age: The ability to metabolize xenobiotics decreases with *age,* which is due to: the reduction in volume of liver, blood flow, and decrease in the activity of some of the metabolizing enzymes. Therefore, the bioavailability of certain xenobiotics is increased in elderly persons, making them susceptible to toxic insult. For example, the bioavailability of propranolol, a drug used in heart patients, is higher in elderly patients. Neonates have lower xenobiotics metabolism capacity, and an adult-like capacity is developed during first 6-8 months of life.

Disease: Liver is the most important site for xenobiotic metabolism, and any disease that decreases the capacity of liver can affect the metabolism of xenobiotics. Patients of hepatitis or cirrhosis have impaired ability to metabolize xenobiotics. These patients thus, have a higher concentration of xenobiotics in blood and making themsusceptible to toxic insult.

5.4.4 Nutritional Factors

Xenobiotic metabolism requires enzymes and cofactors, derived from both macro (protein, carbohydrate, and fats) and micronutrients (vitamins and minerals). Many co-factors such as FAD, NADH, acetyl CoA, glutathione, etc. used by enzymes are derived from vitamins and essential amino acids. Further, many enzymes require metals (such as iron, copper, zinc, nickel, etc.) for catalysis, binding with substrate or for stability of structure. Therefore, deficiencies of nutrients can affect xenobiotics metabolism. In general, severe protein and vitamin deficiency, and starvation can affect xenobiotic metabolism. For example: the toxic metabolites of paracetamol formed in the liver are detoxified by conjugation with glutathione and glucuronic acid. Both these co-substrates are depleted or not synthesized in the required quantity, in case of prolonged starvation.This results in accumulation of toxic metabolite of paracetamol, resulting in hepatotoxicity.

5.4.5 Food Components and Presence of Other Xenobiotics in the Body

Many xenobiotics metabolizing enzymes can be inhibited or induced (i.e. concentration of enzyme can be increased in cells by producing new enzyme molecules by increasing gene expression or translation) by drugs, herbal products, and food components. This leads to reduced or excessive metabolism of other concurrently exposed xenobiotics, and, is one of the reasons of ‘xenobiotic-food’ and ‘xenobiotic-xenobiotic’ interaction (also denoted as ‘drug-food’ and‘drug-drug’ interactions in the field of medicine). Let us see few examples of this phenomenon:

- Certain components in grapefruit juice inhibit intestinal CYP 450 isoform 'CYP3A4' and thus, can increase the bioavailability of many drugs such as felodipine, a drug used to treat hypertension. Similarly, blueberry juice and pomegranate juice can inhibit CYP3A4 and CYP2C9. Also, cruciferous vegetables (cabbage, broccoli, etc.) can induce CYP1A2.These are examples of xenobiotic-food interaction.

- CCl_4 is metabolized to toxic metabolite peroxyl radical ($CCl3OO^•$) in liver by an isoform of CYP 450 'CYP2E1'. This isoform is induced by ethanol. Therefore, alcoholics are more vulnerable to liver injury caused by CCl_4. This is an example of xenobiotic-xenobiotic interaction.

5.4.6 Environmental Factors

Internal body *temperature* and that of surroundings may affect xenobiotics metabolism. At lower body temperature, the activity of metabolic enzymes (or affinity with the substrate) decreases resulting in accumulation and a high concentration of xenobiotics in the body. For example: the concentration of midazolam, a sedative drug increases several folds in plasma under low body temperature, which is due to lowering of CYP3A4 activity due to low temperature.Temperature of surroundings also affects the metabolism of xenobiotics; for example, under low surrounding temperature, the concentration of metabolic enzymes has been found to increase in laboratory rats.

The metabolism of several drugs is altered at *high altitude*due to hypoxic conditions. Under hypoxic conditions at high altitudes, the activity and expression of a few isoforms of CYP 450 increases whereas those of others decreases. For example: under high altitude hypoxic conditions, the metabolism of lidocaine, a local anaesthetic, is decreased thereby increasing its half-life inblood.

The changing *light-dark period* causes diurnal rhythmic changes in physiology, cellular metabolism, hormonal release, sleep cycle (circadian rhythm), etc. The circadian rhythm also affects xenobiotics metabolism in a rhythmic manner.The transcription of many isoforms of CYP 450 changes rhythmically and thus, affects the metabolism of xenobiotics. For example, toxicity of aconitine is higher when it is exposed to laboratory mice during daytime, because the activity of detoxifying enzyme CYP3A11 is less during daytime. Similarly, paracetamol caused more damage to liver when laboratory animals are exposed to the drug in dark period (night).

SAQ 3

a) State whether the following statements are true or false:

i) Vitamin deficiency and internal body temperature of animals may alter xenobiotic metabolism. [True/False]

ii) Polymorphism in xenobiotic metabolizing enzymes does not affect therapy using drugs. [True/False]

b) Fill in the blanks with appropriate words:

i) Components of food affecting metabolism of xenobiotics is an example of .... interaction.

ii) Increase in the concentration or amount of xenobiotic metabolism in cells is called................

5.5 SUMMARY

Let us summarize what we have learnt so far:

- In humans, systemic toxicants mainly get entry in the body by absorption through gastrointestinal tract, lungs and skin. After absorption from the site of exposure, some amounts of a toxicant may undergo-presystemic elimination (mainly due to metabolism). Thus, a fraction of the absorbed toxicant reaches systemic circulation. Once in the systemic circulation, toxicants are carried to various organs through a process known as 'distribution' and simultaneously, the toxicants are also slowly removed or excreted from the body.

- The rate and extent of absorption depends on the physicochemical properties of the toxicants such as lipid solubility and size, etc. In general, lipid soluble low molecular weight chemicals are absorbed well in the intestine by simple diffusion. Water-soluble toxicants use membrane transporters or pass through aqueous pores in the epithelial lining. Although, skin functions as a barrier to the entry of toxicants in the body, it can be easily traversed by lipid-soluble toxicants, whereas water-soluble toxicants get entry through dermal appendages. Absorption of toxic gases and solvent vapours via lungs depends on the blood-to-gas partition coefficient, whereas size and solubility of 'aerosols and particles' are the important factors determining theirabsorption and penetration in the respiratory tree. The extent of absorption is denoted by the parameter 'bioavailability', which is the fraction of absorbed toxicant that reaches systemic circulation.

- Distribution of toxicants to various organs depends on the rate of blood flow to an organ and lipid solubility of toxicants. A lipid-soluble toxicant is widely distributed in the body. Certain toxicants have the property to accumulate in tissues of various organs. These tissues or organs are known as 'storage depots'.The extent of distribution of toxicants in the body is measured by 'volume of distribution'.

- Metabolism of toxicants mainly takes place in liver, enterocytes and lungs. Several enzymes known as 'xenobiotic metabolizing enzymes' modify chemical structures of toxicants and are responsible for detoxification and activation of toxicants. Metabolism of toxicants is affected by genetic, physiological and environmental factors. Marked difference in the pattern of metabolism of toxicants is also observed between species.

- Toxicants are mainly removed or excreted from the body via urine, faeces, and lungs. The rate of removal of toxicants is denoted by the parameter ‘clearance’.

5.6 TERMINAL QUESTIONS

1. Mention various factors which affect absorption of xenobiotics via gastrointestinal tract.

2. Explain, why bioavailability of xenobiotics administered via routes other than intravenous route is low.

3. Genetic polymorphism in xenobiotic metabolizing enzymes causes variation in the toxic response in a population.

4. Discuss, how toxicants get entry into blood via skin.

5.7 ANSWERS

Self-Assessment Questions

1. a) i) False ii) False iii) True iv) True v) True vi) False

   b) i) Millilitres/minute or litres/hour

   ii) Facilitated transport, active transport and via aqueous pores

   iii) Total body burden

2. i) True ii) True

3. a) i) True ii) False

   b) i) Xenobiotic-food ii) Induction

Terminal Questions

1. Factors affecting absorption via gastrointestinal tract includes: physicochemical properties of toxicants (lipid solubility, size, etc.), attributes of the site where absorption takes place: i.e. surface area, properties of epithelium (presence of transporters, thickness of epithelium, etc.) and blood supply in the underlying tissue. Presence of food, various food components, motility of the intestine are other factors.

2. Xenobiotics administered via routes other that intravenous have low bioavailability due to incomplete absorption, and presystemic elimination i.e. metabolic degradation of xenobiotic and excretion from the body (such via bile).

3. Due to polymorphism, several alleles of one or more xenobiotics metabolizing enzymes exist in a population, which produce enzymes that catalyze reactions at different rates. Therefore, the ability to detoxify or activate a xenobiotic may vary amongst individuals of a population, resulting in variation in toxic response.

4. Fat-soluble xenobiotics can cross the stratum corneum layer by simple diffusion and reach dermis. Low molecular weight water-soluble xenobiotics enter dermis via hair follicles and opening of sweat glands at the surface of skin and reach dermis. Upon reaching dermis, toxicants get entry into blood via blood vessels in dermis.

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Unit 6

XENOBIOTIC METABOLISM

Structure

6.1 INTRODUCTION

Xenobiotics are foreign substances or chemicals which may pose serious threat to an organism. To handle this situation, various organisms have evolved a system of enzymes which modify xenobiotics in such a way that these are inactivated or detoxified and quickly removed from the body. These limited number of enzymes with broad substrate specificity act on millions of types of xenobiotics to which organisms or humans are exposed. These so called 'xenobiotic metabolizing' enzymes can catalyze varieties of reactions including oxidations, reductions, hydrolysis, and conjugations. Many of these enzymes play an important role in the metabolism of endogenous molecules also and thus,have an important role in maintaining normal physiology. In this unit, we will learn about xenobiotic metabolizing enzymes and the main reactions catalyzed by them.

Expected Learning Outcomes

After studying this unit, you should be able to:

- Know about xenobiotics
- understand basic strategy by which our body handles various xenobiotics, and importance of xenobiotic metabolism,
- learn about main types of reactions involved in xenobiotic metabolism,
- know main enzyme system of the body involved in xenobiotic metabolism.

6.2 INTRODUCTION TO XENOBIOTICS AND METABOLISM

The term xenobiotic (Greek '*xenos*' meaning: stranger, guest or foreigner) in toxicology is used to denote a substance or chemical that enter the body from an external source. It is not found or produced naturally in the body. The term however, does not indicate whether a chemical is harmful or beneficial to the body or is synthetic or natural. Thus, pesticides, drugs, food additives, plant products, plant and animal poisons, industrial pollutants, etc. are all xenobiotics. It has been estimated that during their lifetime, humans are exposed to about 1-3 million xenobiotics. These come in contact with the body via food, drinking water, air, and administration or application on the body (such as drugs and cosmetics).

'Xenobiotic metabolizing enzyme' system is a defence strategy of the body against non-endogenous chemicals or xenobiotics. Thus, the metabolizing system does not discriminate between beneficial xenobiotics (drugs) and toxicants (e.g. pesticides). The widely presumed significance of existence of xenobiotic metabolism enzyme system is that: i) during metabolism, the chemical structure of xenobiotics entering the body are enzymatically modified, ii) the resultant metabolites of xenobiotics often lose their activity (i.e. a drug or toxicant may become ineffective) and become more water-soluble, and iii) the two main excretory routes of the body (urine and bile) efficiently excrete the hydrophilic metabolites. It may however be noted that in a number of cases, after metabolism, a xenobiotic becomes more active than its original form (e.g. conversion of prodrugs to active drugs and activation of toxicants). Further a few metabolic reactions make xenobiotics more lipid-soluble (acetylation and methylation reactions).

Though humans are exposed to millions of xenobiotics in their lifetime, there are only a few xenobiotic metabolizing enzymes having broad substrate specificities. This means that one enzyme can metabolize several xenobiotics (see Unit 5). Also, a xenobiotic may be metabolized through one pathway or several pathways, and thus, it is a mixture of metabolites that is often excreted in the urine (or other routes). Some of the xenobiotic metabolizing enzymes also have roles in metabolizing endogenous molecules such as steroids and bile acids, vitamins, etc. Though xenobiotic metabolizing enzymes to some extent are found in tissues of several organs, the highest amount is found in liver. Few other organs such as gastrointestinal tract, lungs, and kidney can also contribute significantlyto metabolism of xenobiotics.

The metabolism of xenobiotics is usually carried out in two phases i.e. phase I and phase II. In phase I, functional group/s are introduced or unmasked in xenobiotics through one or more steps. In phase II, bulky water-soluble endogenous molecules (such as glucuronic acid, glutathione) are attached to these functional groups. Reactions of phase I are also called 'functionalization reactions' whereas that of phase II are 'conjugation reactions'. The entire process thus produces a metabolite of a xenobiotic whichis usually inactive and more water-soluble, that is easy to excrete.

The superfamily CYP 450 consisting of 57 enzymes (in humans) is the most important enzyme system responsible for metabolism of xenobiotics. This superfamily is known to metabolize about 70% of all the drugs taken by humans.

Microsomes are vesicular fragments of endoplasmic reticulum. They contain endoplasmic drug metabolizing enzymes CYP450, FMOs and other membrane bound enzymes. Microsomes are obtained by centrifuging homogenate of liver first at low speed, and the supernatant obtained at this step is centrifuged at high speed (to get about 100,000 g). The pellet so obtained represents the microsomal fractionand is widely used in laboratories as source of CYP 450 and other metabolic enzymes to study xenobiotic metabolism.

SAQ 1

a) **State whether the following statements are true or false:**

i) Xenobiotics are the substances which originate outside the body and are harmless. (True/False).

ii) Xenobiotic metabolizing enzymes also metabolize endogenous molecules (True/False).

b) **Fill in the blanks with appropriate words:**

i) Phase II reactions are also known as...................

6.3 PHASE-I REACTIONS

Phase I reactions produce metabolites of xenobiotics which in some cases lose activity or retain original activity; while in some cases, produce more toxic metabolites. Let us discuss below the main phase I biotransformation reactions:

6.3.1 Types of Phase I Reactions

Phase I reactions include, oxidations, reductions and hydrolysis. These reactions introduce new functional groups or unmask already existing groups (–OH, $-NH_2$, –SH, –COOH) in the xenobiotics.The main enzymes responsible for phase I reactions are given in Table 6.1. Note that oxidation reactions are carried out by two groups of enzymes i.e. microsomal and non-microsomal enzymes.

Table 6.1: Main enzymes responsible for catalyzing phase I reactions

Reactions	Main catalyzing enzymes
Oxidations	Dehydrogenases: alcohol dehydrogenase, aldehyde dehydrogenase Oxidases: amine oxidases, aldehyde oxidase (non-microsomal) Peroxidises: myeloperoxidase Oxygenases: cytochrome P450 monooxygenases, flavin monooxygenases (FMO) (microsomal)
Reductions	Aldo–keto reductases Aldehyde dehydrogenase Alcohol dehydrogenase Thioredoxin - dependent enzymes Quinone oxidoreductases
Hydrolysis	Carboxylesterases Cholinesterases Paraoxonase Epoxide hydrolases Peptidases

CYP450 monooxygenase, the most important enzyme of phase I, is involved in oxidation reactions of over 90% of the drugs taken by humans. Let us see below the molecular details and nature of reaction catalyzed by CYP450.

6.3.2 Cytochrome P-450 Monooxygenase

Cytochrome P450 (CYPs), a group of monooxygenases is responsible for metabolism of a large number of xenobiotics, and thus, plays a key role in detoxification of toxicants; and affects the duration of action of drugs in the body. In many cases, actions of CYPs lead to activation of xenobiotics that causes toxicity. CYPs also play a role in metabolism of endogenous molecules such as steroids, fatty acids, bile acids, fat soluble vitamins A and D, etc. A mutation in some CYP genes leads to failure of development of normal male or female characteristics (Fig. 6.1).

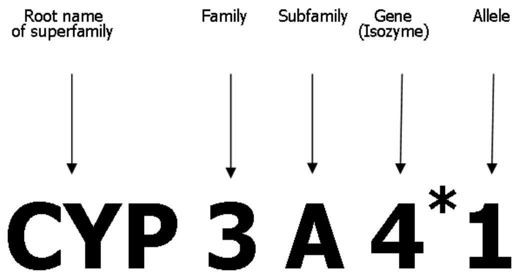

Fig. 6.1: Nomenclature of CYP450.

CYPs are found in all the tissues of the body, however, liver has the highest level of this enzyme, and it is also found in significant amount in enterocytes, kidneys and lungs. Intracellularly, highest amount of CYPs is found in membranes of endoplasmic reticulum; however, it also occurs in the inner membrane of mitochondria.

CYPs are heme containing proteins and get their name from the fact that a complex formed between CYP and carbon monoxide has absorption maxima at 450 nm. In humans, CYP450 superfamily has 18 families, 31 subfamilies and 57 genes, coding for 57 isoforms of the enzyme CYP450 monoxygenase. The name of individual isoforms of CYP has identifiers of family, subfamily and gene. Refer to figure 1 for nomenclature of a CYP enzyme. The main CYPs responsible forthe metabolism of xenobiotics belong to the families 1, 2 and 3. Genes coding for isozymes CYP2A6, CYP2B6, CYP2C8, CYP2D6, CYP3A5, CYP1A2, CYP2C9, CYP2C19 show high genetic polymorphism i.e. exist in several allelic forms in the human population. These are responsible for most of the observed inter-individual variation in xenobiotic metabolism and thus,the toxic response (see Unit 5).

The overall reaction catalyzed by CYP 450 monooxygenases can be given as:

$$\underset{\text{Substrate}}{\text{R-H}} + O_2 + \text{NADPH} + H^+ \longrightarrow \underset{\text{Product}}{\text{R–OH}} + H_2O\ \text{NADP}^+$$

In the above reaction, NADPH acts as electron donor, which donates electron to catalytic centre (heme) of CYP 450 via FAD/FMN-containing P450 reductase. As it is apparent from the equation, products formed are hydroxylated derivatives of original xenobiotics. This however, is not always the case, because of rearrangement taking place in the molecules. Thus, the final products are variable and include: epoxides of original xenobiotics; O–,S– or N– dealkylated products; S–and N– oxygenated products; deaminated products; cleavage of ester bonds; dehalogenated products, etc. Thus, on the basis of final products, reactions of CYP 450 can be of the following types:

i) Hydroxylation

ii) Epoxidation

iii) Oxidative deamination

iv) Dealkylation

v) Oxygenation (N– and S– oxygenation)

vi) Oxidative dehalogenation

vii) Reductions

viii) Isomerisation

Let us see examples of some of these reactions!

i) *Hydroxylation*: Hydroxyl group can be introduced by CYPs in both aromatic and aliphatic hydrocarbons. For example, benzene can be hydroxylated in liver to hydroquinone which is converted to reactive phenoxy radical in the bone marrowby peroxidases resulting in bone marrow suppression and leukaemia (Fig. 6.2).

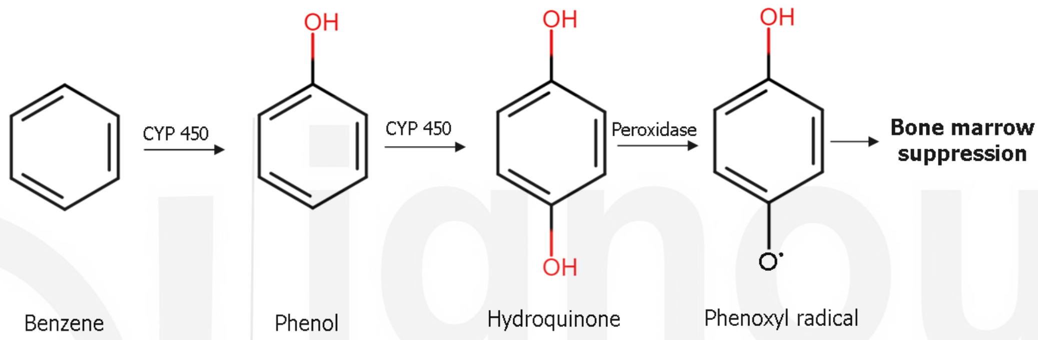

Fig. 6.2: Hydroxylation of benzene by CYP450 and mechanism of bone marrow suppression.

ii) *Epoxidation:*Xenobiotics with double bonds can undergo epoxidation by CYPs (Fig. 6.3). Epoxides are also formed as intermediates during hydroxylation reactions. Epoxides are toxicologically important products as these are highly reactive and can covalently react with cellular components if not subsequently detoxified; and can cause carcinogenesis and necrosis of cells.

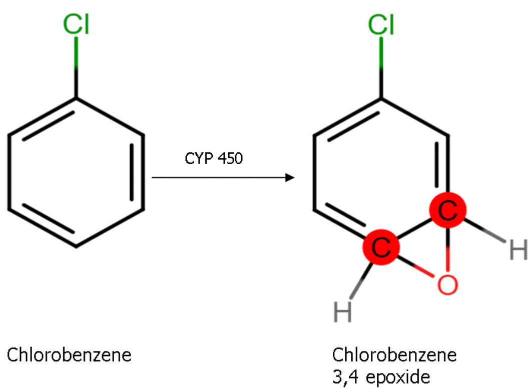

Fig. 6.3: Epoxidation of chlorobenzene by CYP450.

iii) *Oxidative deamination:* In this reaction, oxygenation of the carbon adjacent to the amine group is followed by rearrangement in the molecule resulting in loss of amine group as ammonia. Fig. 6.4, depicts oxidative deamination of drug amphetamine.

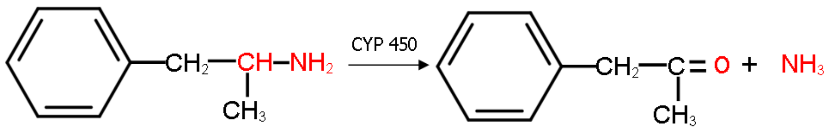

Fig. 6.4: Oxidative deamination of amphetamine by CYP450.

iv) *Dealkylation:* In this reaction, oxygenation of a substrate having, O–, N– or S–alkyl groups is followed by release of an aldehyde (Fig. 6.5).

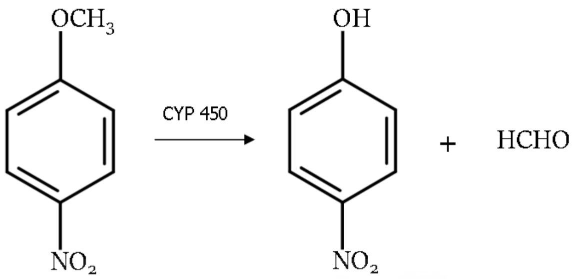

Fig. 6.5: Dealkylation (*o*-dealkylation) of *p*-nitroanisole by CYP450.

v) *Oxygenation:*In this reaction, an oxygen atom is incorporated at N– and S– or other heteroatoms of the substrate. N–oxidation of aromatic substrate by CYPs results in hydroxylamines (Fig. 6.6).

$$R\text{–}NH_2 \xrightarrow{\text{CYP 450}} R\text{–}NHOH$$

Substrate Product

Fig. 6.6: N–oxidation of an amine by CYP450.

vi) *Reductions:* Under low oxygen conditions in liver, CYPs can catalyze reduction reactions such as *reductive dehalogenation*. For example, in liver, halothane (an anaesthetic) can be reduced by CYPs to a carbon-centric radical which can react with proteins and constituents of cell membrane causing liver damage. However, under normal oxygen conditions, CYPs oxidize halothane (*oxidative dehalogenation*) to a reactive product trifluoroacetylchloride (also hepatotoxic). Similarly, one electron reduction of CCl_4 by CYPs produces trichloromethyl radical ($^{\bullet}CCl_3$) which causes hepatotoxicity (Fig. 6.7).

$$CCl_4 \xrightarrow{\text{CYP 450}} {}^{\bullet}CCl_3$$

Carbontetrachloride Trichloromethyl radical

Fig. 6.7: Reductive dehalogenation of carbon tetrachloride by CYP450.

vii) *Isomerisation*: A few isoforms of CYPs are known to catalyze isomerisation of substrates. For example: isoforms 'CYP5A1' and 'CYP8A1', convert PGH_2 into 'TXA'$_2$ (thromboxane A_2)'and 'PGI'$_2$

(prostacyclin)', and are also known as 'thromboxane synthase' and 'prostacyclin synthase' respectively. These isoforms do not require NADPH (Fig. 6.8).

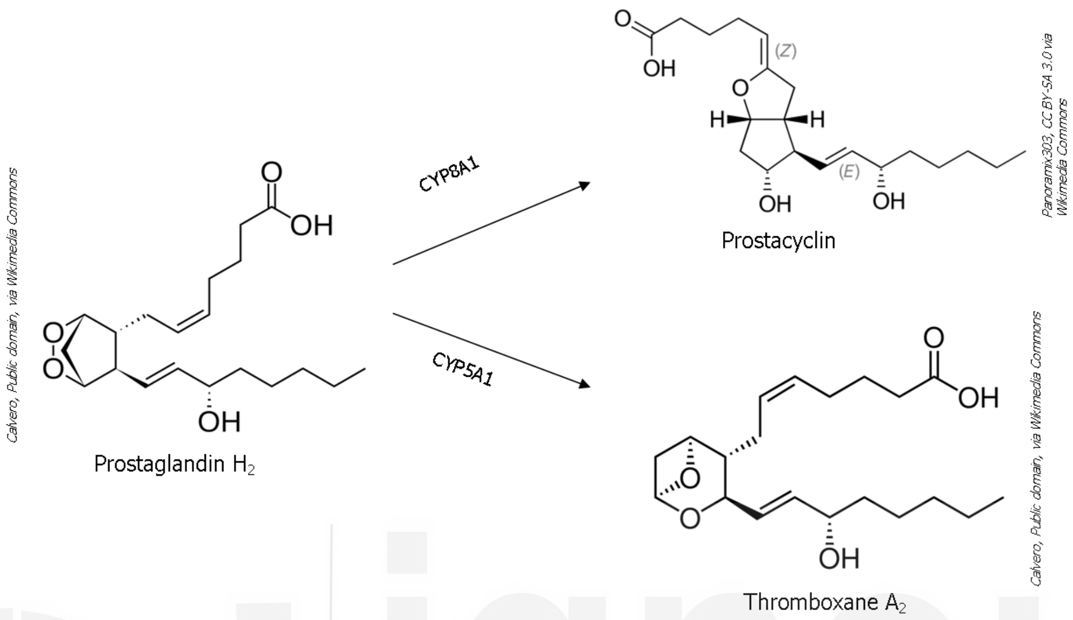

Fig. 6.8: Isomerisation of PGH_2catalyzed by CYP450.

SAQ 2

a) State whether the following statements are true or false:

i) Oxidation of xenobiotics is the most common reaction of phase I (True/False).

ii) CYP 450 produces only hydroxylated metabolites of xenobiotics (True/False).

iii) Under high oxygen conditions, CYPs can also perform reduction reactions (True/False).

iv) The reaction centre of CYP 450 receives electrons form reduced glutathione (True/False).

v) Xenobiotics with double bonds can be converted to epoxide derivatives by CYPs (True/False).

b) Fill in the blanks with appropriate words:

i) Conversion of benzene to hydroquinone by CYPs is example of ....reaction.

ii) Other than endoplasmic reticulum, significant CYP activity is found in..........

6.4 MICROSOMAL AND NON-MICROSOMAL OXIDATION SYSTEMS AND THEIR ROLE IN XENOBIOTIC METABOLISM

Refer to the table 1 above, wherein enzymes involved in oxidation of xenobiotics are divided into two groups viz. 'non-microsomal' and 'microsomal'. This is based on the location of the enzymes within the cells. Non-microsomal enzymes are located mainly in cytosol, mitochondria, peroxisomes, nucleus and cell membrane; whereas microsomal enzymes are located in endoplasmic reticulum (see above). In endoplasmic reticulum, almost all the oxidation reactions are carried out by the two enzyme systems viz. CYPs and FMOs, and the two enzymes together catalyze most of the oxidation of xenobiotics in the body. CYPs and FMOs catalyze similar reactions, however, there exists substrate selectivity between the two. Non-microsomal oxidations are mainly carried out by alcohol dehydrogenase, aldehyde dehydrogenase, xanthine oxidoreductases, amine oxidase and aldehyde oxidases. Most non-microsomal oxidative enzymes are not inducible or less so, as compared to CYPs. Therefore, the phenomenon of xenobiotic-xenobiotic and food-xenobiotic (see Unit 5) is not common with non-microsomal enzymes. Further, because non-microsomal enzymes are less inducible, the phenomenon of 'tolerance' is mostly due to induction or microsomal enzymes (CYPs in particular, see below).

6.5 INDUCTION OF CYTOCHROME P450

Enzyme induction is the process by which the activity of xenobiotic metabolizing enzymes is increased by the same xenobiotic which it metabolizes, or other xenobiotics. Induction is an adaptive process by which during the period of high exposure to toxicants, the body temporarily increases metabolizing enzymes, thus removing toxicants from the body at a faster rate. Enzyme induction can also have several other consequences, for example: development of tolerance to drugs, making drugs ineffective or toxic, and increasing toxicity of toxicants. Tolerance (see Unit 1 for definition) to drugs and xenobiotics develops due to their increased metabolism by the induced enzyme, for example: ethanol increases its own metabolism by inducing the isoform CYP2E1. Induction of CYP2E1 also makes an alcoholic sensitive to CCl_4 toxicity, because the latter is activated by CYP2E1. In the above example, induction of CYP2E1 on one hand prevents ethanol toxicity by rapid metabolization, and on the other hand, increases sensitivity to another toxicants (i.e. CCl_4 in the above example).

It is not just CYPs, many other xenobiotic metabolizing enzymes (such as, UDP-glucuronosyltransferases, sulfotransferases, glutathione-S-transferase, etc.) are also inducible. The mechanism of induction of metabolizing enzymes includes: i) an increased transcription of genes coding for enzymes, and ii) increase in stabilization of mRNA and enzyme molecule from degradation. The former is the most common mechanism which we shall discuss in the following paragraph.

Induction of xenobiotic metabolizing enzymes at transcription level is mediated by certain proteins denoted as ‘xenosensors’. Aryl Hydrocarbon Receptor (AhR), PXR (Pregnane X Receptor), CAR (Constitutive Androstane Receptor), and PPARα (Peroxisome Proliferator–Activated Receptor Alpha) to name a few are xenosensors. These xenosensors have a ligand binding domain which specifically binds to xenobiotics. The xenobiotic-xenosensor complex, after binding with a few accessory proteins, finally binds to DNA and increases the transcription of genes coding for metabolizing enzymes. Let us explain the process by using the example of a xenosens or AhR. This xenosensor binds to several lipid-soluble toxicants (such as TCDD, PAHs, etc.), drugs (such as omeprazole, lansoprazole, etc.) and endogenous molecules (such as metabolites of tryptophan, arachidonic acid, etc.). AhR induces several phase I and II enzymes. AhR remains in the cytoplasm when not bound to a ligand. After binding with a ligand, it dissociates from accessory proteins and is translocated to the nucleus where it forms a complex with a protein called ARNT (aryl hydrocarbon receptor nuclear translocator). The AhR-ARNT complex binds to DNA and increases transcription of xenobiotic metabolizing enzymes (Such as CYPs, UGTs, SULTs, etc.).

Since xenobiotic metabolising enzymes also metabolize several endogenous molecules, induction of these enzymes by toxicants can affect metabolism of endogenous molecules and disturb homeostasis.

6.6 PHASE-II REACTIONS

As we have discussed in Unit 5 that water-soluble xenobiotics or their metabolites are efficiently excreted via our main excretory routes (urine and via bile in faeces). By introducing or unmasking functional groups, phase I reactions slightly increase water solubility of xenobiotics as well as provide site/s where during phase II reactions, certain water-soluble endogenous molecules (Fig. 6.9) can be attached or ‘conjugated’. The resultant conjugates of xenobiotics, which are now highly water-soluble,are transported actively by the membrane transporters of hepatocytes into the bile canaliculi to be excreted in faeces. A similar situation occurs in kidney, where conjugatesof xenobioticsare also efficiently excreted. This is because conjugates, being water-soluble, are not reabsorbed in the blood from glomerular filtrate, andare also actively secreted in the lumen of kidney tubules to be excreted in the urine.

It is to be noted that not all phase II conjugation reactions increase water-solubility of xenobiotics; a few reactions (such as methylation and acetylation) make xenobiotics more fat-soluble.

Below are the main phase II biotransformation reactions:

i) Glucuronidation (conjugation of xenobiotics with glucuronic acid)

ii) Sulfonation (conjugation/introduction of sulfonate group in xenobiotics)

iii) Glutathione conjugation (conjugation of xenobiotics with glutathione)

iv) Amino acid conjugation (conjugation of xenobiotics with amino acids such as glycine, taurine, glutamine, etc.)

v) Acetylation (introduction of an acetylgroup in the xenobiotic)

vi) Methylation (introduction of a methyl group in the xenobiotic)

All the above reactions are catalyzed by various enzymes which use high energy cofactors as carriers (Fig. 6.9) of activated endogenous molecules to be conjugated/introduced in the xenobiotic (in case of glucuronidation, sulfonation, acetylation and methylation) or use activated xenobiotics (in case of amino acid conjugation).

Glucuronidation and sulfonation are the major pathways of phase II xenobiotic metabolism.

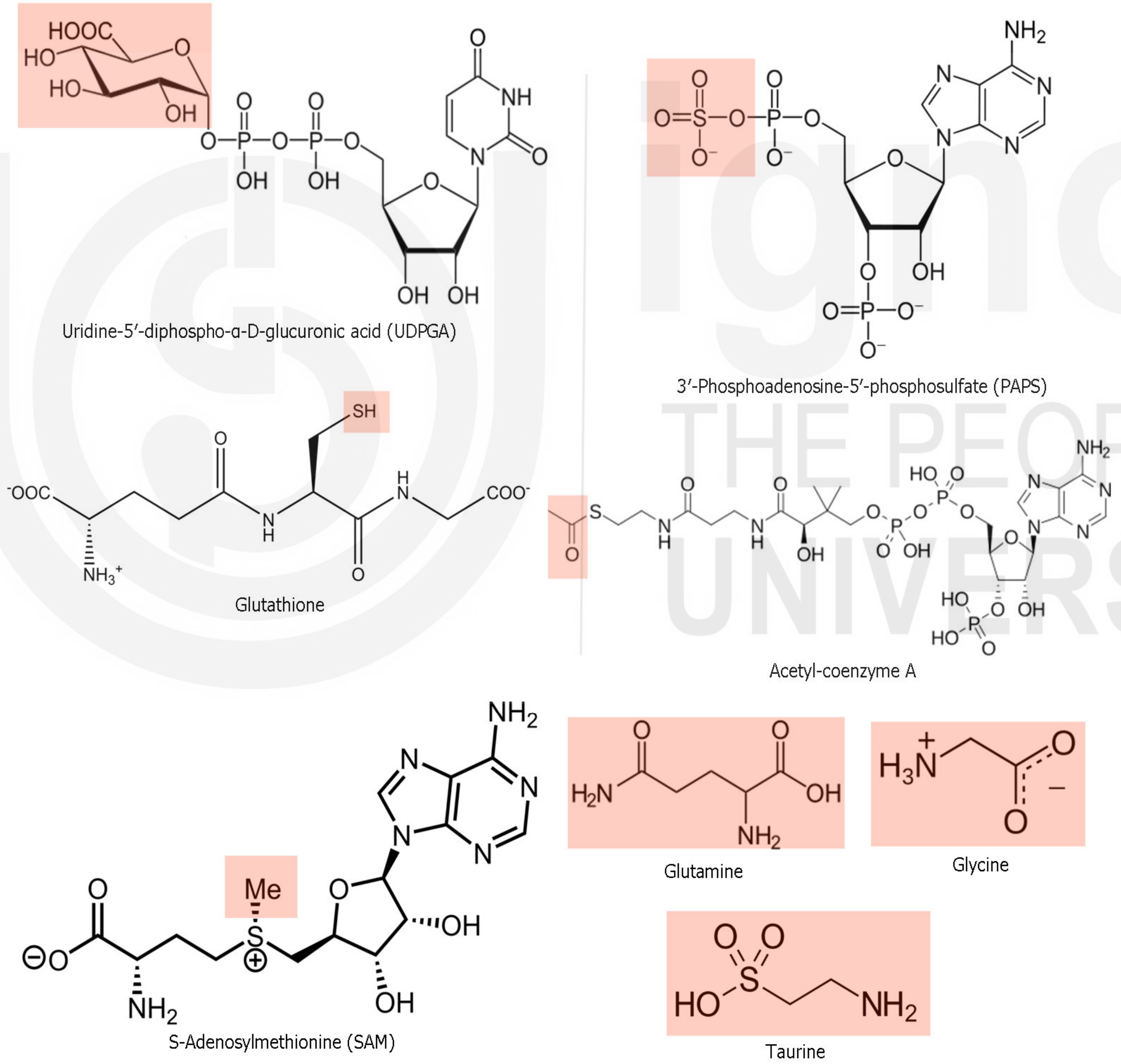

Fig. 6.9: Cofactors involved in phase II conjugation reaction. The group that reacts with the xenobiotics or is transferred to their functional groupsare enclosed in red boxes.

(All images courtesy: https://commons.wikimedia.org/wiki)

i. *Glucuronidation:* This reaction is catalyzed by 'uridine diphosphate-glucuronosyl transferases (UGTs)' located mainly in endoplasmic reticulum of liver and several other tissues. In this reaction, the cofactor 'UDP glucuronic acid (UDPGA)' provides activated glucuronic acid to be introduced tothe xenobiotics. The sitewhere glucuronic acid is introduced in xenobiotics are nucleophilic atoms such as 'O', 'N' and 'S'. Thus alcohols, phenols, carboxylic acid, amines and thiol group containing xenobiotics readily undergo glucuronic acid conjugation (see Fig. 6.10).

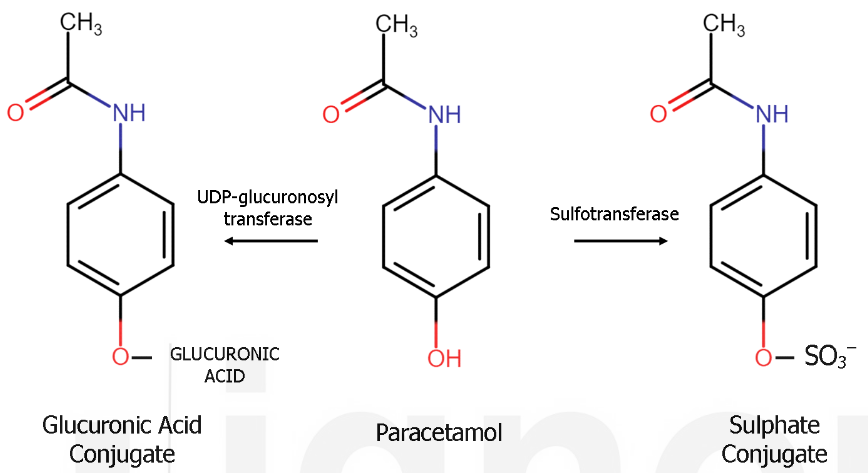

Fig. 6.10: Glucuronidation and sulfonation of paracetamol.

ii. *Sulfonation:* This reaction is catalyzed by enzymes 'sulfotransferases (SULTs)' found in cytoplasm and uses '3′-phosphoadenosine-5′-phosphosulfate (PAPS)' as cofactor for donating sulfonate group. Several xenobiotics which undergo glucuronidation are also conjugated with sulfonate (see Fig. 6.10).

iii. *Glutathione conjugation:* This reaction also called 'glutathionylation' is catalyzed by 'glutathione S-transferase (GST)' found in mitochondria, cytosol and microsomes. In this reaction, the sulfhydryl group (or thiolate) of glutathione is conjugated at 'electron deficient' carbon atoms (such as those linked to 'O', 'N' and 'S' atoms) or other electrophilic atoms of xenobiotics. As shown in Fig. 6.11, chlorobenzene undergoes phase I reaction and is converted to a reactive epoxide derivative by CYP450, which is conjugated with glutathione by glutathione S-transferase, resulting in its detoxification and an increase in water-solubility of the metabolite.

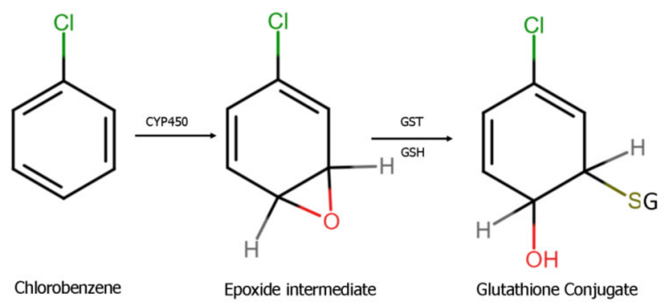

Fig. 6.11: Glutathione (SG) conjugation of an epoxide derivative of chlorobenzene.

iv. *Amino acid conjugation:* In this reaction, amino acids such as glycine, glutamine, ornithine, taurine, serine, etc. are conjugated with the xenobiotic. There are two pathways of amino acid conjugation; in the first, the –COOH group of xenobiotic is activated by coenzyme A. The activated –COOH group is then conjugated with amino group of amino acid by enzyme 'N-acyltransferase' (Fig. 6.12).In the second pathway, amino acids are activated by 'aminoacyl-tRNA synthetases',–COOH group of which is conjugated to an aromatic hydroxylamine xenobiotic.

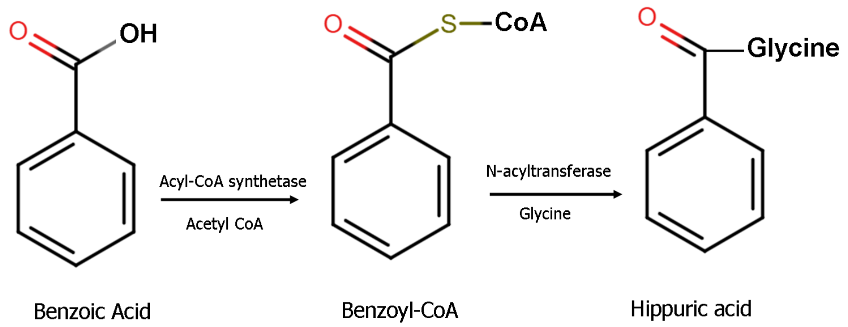

Fig. 6.12: Conjugation of glycine with benzoic acid

v. *Acetylation:* In this reaction, an acetyl group is transferred from the cofactor 'acetyl coenzyme A (acetyl-CoA)' to the nitrogen atoms of amine and hydrazine groups in xenobiotics. The reaction is catalyzed by 'N-acetyltransferases (NATs)' found in the cytoplasm and mitochondria. After acetylation, xenobiotics with amineand hydrazine groups are converted to their amide and hydrazide derivatives. Acetylation reaction has no significant effect onincreasing water-solubility of xenobiotics and hence, their excretion via urine and bile (Fig. 6.13).

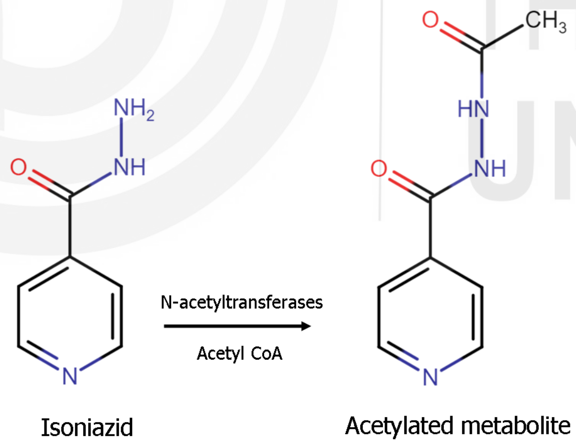

Fig. 6.13: Acetylation of antitubercular drug isoniazid by NAT.

vi. *Methylation:* In this reaction, a methyl group is transferred from the cofactor 'S-adenosylmethionine (SAM)' to the electron rich atoms ('O', 'N' or 'S') in xenobiotics with the help of 'methyltransferases' found in cytosol and microsomes.The functional groups in xenobiotics where methylation takes place include phenolic group, amine, and thiol. In most cases, after methylation, the water-solubility of xenobiotics decreases. In some cases however, methylation causesatoms (such as N or S) to acquire charge and increases the water solubility of xenobiotics (Fig. 6.14).

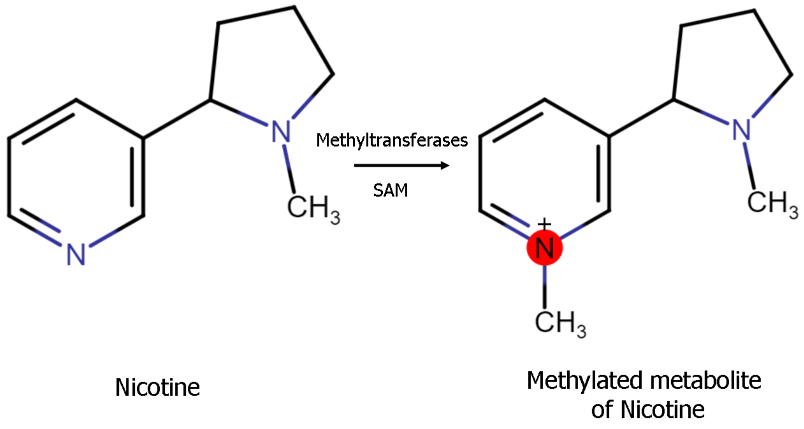

Fig. 6.14: Methylation of nicotine by methyltransferases.

SAQ 3

a) State whether the following statements are true or false:

i) In most cases, methylation of xenobiotics produces water-soluble metabolites which can be easily excreted in urine (True/False).

ii) S-adenosylmethionine acts as cofactor for acetylation reaction (True/False).

iii) Induction of a xenobiotic metabolizing enzyme by a xenobiotic may cause xenobiotic-xenobiotic interaction (True/False).

b) Fill in the blanks with appropriate words:

i) The cofactor.................... is used to donate glucuronic acid to xenobiotics during glucuronic acid conjugation reaction.

ii) .... and .... are the major phase II xenobiotic metabolizing reactions.

6.7 SUMMARY

Let us summarize what we have learnt so far:

- Humans are exposed to a large numbers of various xenobiotics. A xenobiotic is a substance foreign to the body i.e. not produced naturally in the body and, includes both beneficial (drugs) as well as toxic substances.

- To tackle a threat from xenobiotics, organisms have evolved strategies: i) to quickly detoxify the xenobiotics by modifying their chemical structure as they enter the body and ii) make them water-soluble so that their metabolites can be removed from the body efficiently via urine and bile. The xenobiotic metabolizing enzyme system together with the excretory system of the body performs these tasks.

- Though organisms are exposed to a vast variety of xenobiotics in their life time, there are only limited numbers of metabolizing enzymes which have broad substrate specificity. These enzymes are located in every organ of the body, with higher amounts in liver, enterocytes, lungs and kidneys.

- The reactions catalyzed by xenobiotic metabolizing enzymes can be divided into two phases viz. phase I and phase II. Phase I reactions introduce new functional groups or unmask already present functional groups in xenobiotics. In phase II reactions, xenobiotics with functional groups are conjugated with certain water-soluble endogenous molecules (glucuronic acid, glutathione, sulfonate group, etc.). The resultant water-soluble metabolites are easily excreted in urine and bile.

- Phase I reactions include oxidations, reductions and hydrolysis. Each of these reactions is catalyzed by separate groups of enzymes. Oxidations are the most important phase I reaction. The enzymes catalyzing phase I oxidations can be grouped into microsomal and non-microsomal enzymes. The superfamily of enzymes CYP 450, located mainly in microsomes is responsible for the oxidation of most of the xenobiotics entering the body. The activity of several isoforms of this group of monooxygenases can be induced by xenobiotics and components of food.

- CYPs catalyze substrate hydroxylation reaction, however, due to rearrangement in the xenobiotics during enzymatic reaction, the final products formed are of several types including: epoxides, heteroatom oxygenated products, dealkylated and deaminated products, dehalogenated products, etc.

- Glucuronic acid conjugation and sulfonation are the main phase II reactions catalyzed by glucuronosyl transferases and sulfotransferase respectively. The phase II reactions–acetylation and methylation are exception to the general rule, which instead of increasing, decrease water-solubility of xenobiotics.

6.8 TERMINAL QUESTIONS

1. Discuss how Phase I and II reactions contribute to the protection of body against toxicants.

2. Discuss importance of CYP 450 in the metabolism of toxicants.

6.9 ANSWERS

Self-Assessment Questions

1. a) i) False ii) True

   b) i) Conjugation reactions

2. a) i) True ii) False iii) False iv) False v) True

   b) i) Hydroxylation

ii) Mitochondria

3. a) i) False ii) False iii) True

   b) i) UDP glucuronic acid ii) Glucuronidation and sulfonation

<u>Terminal Questions</u>

1. The main excretory routes of our body efficiently remove water-soluble toxicants or xenobiotics. After absorption, phase I reactions, by introducing certain functional groups in xenobiotics make them slightly water-soluble as well as furnish sites where water-soluble endogenous molecules can be conjugated in phase II. After phase II conjugation reactions, xenobiotics usually lose their toxic activity and become water-soluble, thus suitable for excretion.

2. CYP450 is the most abundant and versatile group of xenobiotic metabolizing enzymes in the body. It can metabolize over 70% of the xenobiotics to which we are exposed. Although only 57 isozymes of CYP 450 are found in humans, their wide substrate preference enables them to metabolize vast number of xenobiotics. The highest concentration of CYPs is found in liver, and high blood flow in the liver ensures that toxicants are delivered continuously to the metabolizing enzymes. Many CYP isozymes are inducible and thus,during the period of high exposure to toxicants, body temporarily increases metabolizing enzymes and removes toxicants from the body at a faster rate.

6.10 FURTHER READINGS

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Unit 7

GENETIC BASIS OF TOXICITY

Structure

7.1 INTRODUCTION

In the Unit 3, we learnt that the level of response (to toxicants and Adverse Drug Reactions 'or' ADRs to drugs) to a given dose of 'toxicant' or 'drug' (xenobiotics) may vary among different individuals of a population. Furthermore, some individuals (called 'sensitive individuals') in the population require a lower dose to show same level of response than that is required bymany other individuals (called 'resistant individuals'). Other than this quantitative difference in the response, a small number of individuals in a population may also show the type of toxic responses which are qualitatively different from the responses shown by majority of the individuals (allergic response and idiosyncratic response). These aforementioned'inter-human' (also known as'inter-individual'/ 'intra-specific') variation in response to toxicants (or drugs) is caused by genetic polymorphism (existence of two or more alleles of genes) in the population, ageand life stage of individual, gender, co-existing disease, lifestyle (smoking, alcohol, stress, exercise, etc.), nutritional status, etc. (Fig. 7.1). Polymorphism in the genes coding for proteins involved in xenobiotic 'transport' (membrane transporters, channels, carriers, etc.), 'metabolism' (xenobiotic metabolic enzymes), and 'target molecules' (such as those with which a xenobiotic interacts to show effect, e.g. receptors, enzymes, channels, etc.) accounts for most of the observed inter-human variations in response to toxicants/drugs.

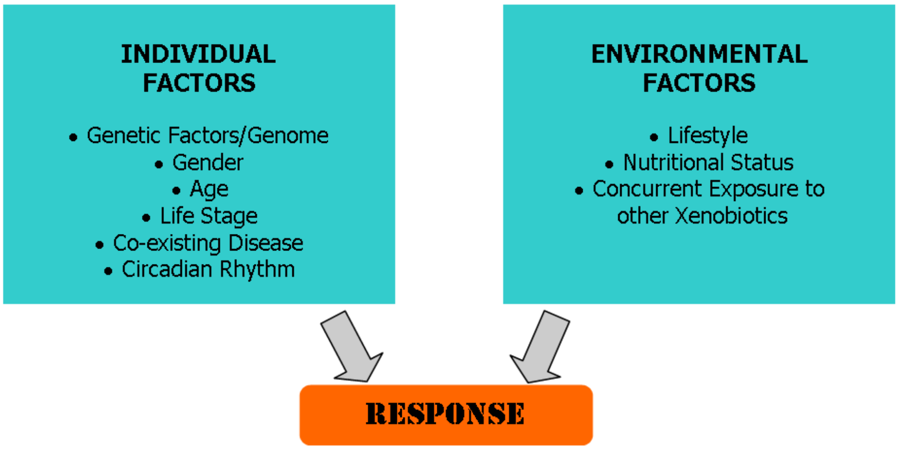

Fig. 7.1: Factors modifying response to toxicants/drugs.

In the Unit 5, we briefly discussed how polymorphism in the genes coding for xenobiotic metabolizing enzymes causes variation in toxic responses in a population. In the following section, we will learn more about it, and also, variation in toxic responses due to polymorphism in genes coding for xenobiotic transporters and receptors.

Expected Learning Outcomes

After studying this unit, you should be able to:

❖ know the main reasons for inter-human variation in response to toxicants and drugs,

❖ know how genetic polymorphism can affect the outcome of toxic and drug response,

❖ know basics of molecular markers and their uses in the field of pharmacogenetics and pharmacogenomics.

7.2 GENETIC POLYMORPHISM AND VARIATION IN XENOBIOTIC RESPONSE

7.2.1 Variation in Response due to Polymorphism in Xenobiotic Metabolizing Enzymes

Polymorphism in the xenobiotic metabolism has been long recognized and a well-studied cause of inter-human variation in response to toxicants/drugs. Polymorphism or genetic variation in genes due to mutations may alter the amino acid/s in the xenobiotic metabolizing enzymes, which may reduce or increase the activity of the enzyme molecule. In the simplest scenario (where a xenobiotic is metabolized by only one enzyme), it can be assumed that individuals in a population homozygous for the alleles producing defunct enzyme molecules will not be able to metabolize (or degrade) certain xenobiotics efficiently. Such individuals wouldaccumulate xenobiotics in the blood and are at a risk to show toxic response. In other words, a dose inadequate to show toxic effects in most other individuals (harbouring normal alleles) is likely to cause toxicity in susceptible individuals.

We may categorize individuals in a population as; Poor Metabolizers (PMs), Intermediate Metabolizers (IMs), Extensive Metabolizers (EMs), and Ultra-rapid metabolizers (UMs). These individuals harbour 'no functional alleles', 'one functional/one dysfunctional allele', 'both functional alleles', and 'one or both alleles with gain in function', respectively. Table 1, lists a few polymorphic xenobiotic metabolic enzymes known to cause variation in response to toxicants/drugs in the human population.

Let us discuss this phenomenon with a few examples:

1. The isoform of CYP 450 i.e. CYP2C9 exists in more than 30 allelic forms. Two of the alleles (designated as CYP2C9*2and CYP2C9*3) produce enzyme molecules in which substitution mutations results in reduced catalytic activity. The CYP2C9*2 allele is more prevalent in Caucasians than in Asians. The drug warfarin,which has a low 'therapeutic index'is metabolized by CYP2C9. Individuals in a population homozygous for CYP2C9*2 allele are prone to warfarin overdosing and resultant toxicity (such as bleeding). Therefore, a reduction in therapeutic dose is recommendedin individuals who are PMs for warfarin.

2. The enzyme Aldehyde Dehydrogenase (ALDH) metabolizes aldehyde group in xenobiotics into carboxylic group. The isoform ALDH2 metabolizes simple aldehydes such as acetaldehyde. An allele of ALDH (ALDH2*2) producing non-functional enzyme molecules (due to a point mutation) has been reported to be prevalent in individuals of Asian origin. Some individuals of this group also harbour an allele of Alcohol Dehydrogenase 'or' ADH (ADH1B*2) which rapidly converts ethanol to acetaldehyde. Individuals harbouring both of these alleles rapidly convert ingested ethanol to acetaldehyde, but the acetaldehyde is slowly converted to acetate. Rapidly accumulating acetaldehyde in the blood stimulates release of catecholamines (nor-epinephrine, epinephrine) which causes immediate toxic effects including; nausea, headache, palpitations, abnormal blood pressure and heart beat, facial flushing.

$$\text{Ethanol} \xrightarrow{\text{ADH}} \text{Acetaldehyde} \xrightarrow{\text{ALDH}} \text{Acetic Acid}$$

3. The enzyme N–acetyltransferase (isoforms: NAT1 and NAT2) catalyzes acetylation of several drugs. Genetic polymorphism in NAT2 is known to cause toxicity by the drugs which are acetylated (or metabolized) by this enzyme.The antitubercular drug isoniazid is less efficiently metabolized and cleared from

Therapeutic Index (TI)

This parameter is a measure of safety of drugs. It compares the dose which produces beneficial effects with the dose which produces toxic effects. It can be calculated as:

TI= LD50 / ED50

Where LD 50 is the 'Lethal Dose' which can kill 50% of the experimental animals and ED 50 is the 'Effective Dose' which produces therapeutic effect in 50% of the experimental animals. These values can be obtained from the Quantal Dose Response Curves (see Unit 3).

It is evident from the formula that a safer drug should have higher 'TI' value. A drug with a low or narrow TI carries a risk of overdosing, and a person receiving such drugs should be closely monitored for any signs of drug toxicity.

the blood in individuals harbouring allele of NAT2 which slowly metabolizes the drug. This leads to a higher concentration of drug in the blood in these individuals compared to other who have normal alleles, putting the former group of individuals at risk of isoniazid toxicity.

Table 7.1: A few xenobiotic metabolizing enzymes and their polymorphic isoforms.

1. Cytochrome P450 (CYP) CYP2D6 CYP2C8 CYP2C9 CYP2C19 CYP2A6 CYP2B6 2. Aldehyde Dehydrogenases (ALDH) ALDH2 3. Alcohol Dehydrogenases (ADH) ADH2 ADH3 4. Glutathione S□transferases (GST) GSTM1 GSTT1 GSTZ1 GSTP1	5. UDP–glucuronosyltransferases (UGT) UGT1A1 6. Sulphotransferases (SULT) SULT1A1 SULT1A3 SULT1C2 SULT2A1 SULT2A3 SULT2B1 7. Thiopurine Methyltransferase TPMT 8. N-acetyltransferase NAT2 NAT1

Genetic Polymorphism

The ‘human nuclear genome’ in all the humans is consisted of 24 chromosomes (22 autosomes + X, Y sex chromosomes) and contains ~ 3 billion nucleotide pairs. The genomes of any two individual are ~ 99.6 % identical ‘or’ in other words only ~0.4% different. It is the difference of ~0.4% that makes each of as a unique individual. The differences (or genomic variations) in our genomes mainly occur in the form of: i) variations in a single nucleotide at several places in the chromosomes (Single Nucleotide Variations or SNVs), ii) insertion/deletion of few nucleotides and, iii) variations in the numbers of sets of ‘unique repeating nucleotide sequence’ in a stretch of chromosome (such as Copy Number Variations or CNVs). No two individuals have same pattern these genomic variations. Genomic variations make certain individuals overall prone to diseases/conditions such as hypertension, diabetes, obesity, etc. Genomic variations are also responsible for the observed differences in response to the drugs and toxicants among individuals of a population. If the nucleotide sequence at a particular location (locus) on a chromosome is variable and each of these variant (or alleles) occurs with a frequency of 1% or higher in a population in, those case the locus is said to be polymorphic i.e. harbouring more than one type nucleotide sequence. This is known as ‘genetic polymorphism’. A polymorphic gene is the one which exists in more than one allelic forms (variants) with a frequency of 1% or higher in a population.

The most prevalent type of genomic variation that exists in our genome is SNVs. Since many of these variants exist with a frequency of 1% of higher, they are denoted as ‘Single Nucleotide Polymorphisms (SNPs)’. SNPs are found throughout the human genome and the average density of SNP in the genome is about 1 (SNP) per 1000–1300 nucleotides. SNPs may lie in the ‘coding regions’ or the ‘regulatory regions’ of genes (such as promoters). SNPs occurring in the coding regions in the genes may not result in replacement of amino acid because of degeneracy of codons (synonymous substitution). Alternatively replacement of the original amino acids with the one with different physicochemical property often results in the loss or diminished protein function (non-synonymous substitution). SNPs in ‘non-coding’ but regulatory regions of genes (such as promoters) may result in alteration in the level of protein expression. SNPs lying in the other parts of non-coding genome (such as those considered ‘junk DNA’) does not cause any loss or gain of biological function in organisms.

7.2.2 Variation in Response due to Polymorphism in Xenobiotic Transporters

Cell membranes of all the cells in our body are embedded with several membrane transport proteins. These transporters enable hydrophilic endogenous molecules and xenobiotics to cross cell membranes (Table 2). Polymorphism in the genes coding for these transportersis another reason for the observed inter-human variation in response to atoxicant/drug.The polymorphism in transporters may cause inter-human differences in the ability to absorb xenobiotics, their distribution in the body, entry into the target cells, and excretion by the kidneys (tubular secretion) and liver (secretion of xenobiotics in bile) or other routes.

Xenobiotic transporters are integral membrane proteins, some of which use energy by hydrolyzing ATP to transport molecules across cell membrane.

Other transporters use ion gradient or, concentration gradient of xenobiotic across cell membrane to transfer xenobiotics from one-side-to other side of the cell membrane. Xenobiotic transporters can be grouped into three superfamilies, viz.ATP-Binding Cassette (ABC), Solute-Linked Carrier (SLC), and Solute Carrier Organic Anion (SLCO) superfamilies. The ABC transporters are 'primary active' transporters which use ATP to transport xenobiotics across cell membrane. The members of SLC superfamily are 'secondary active' transport proteins, whereas the mode of transport by SLCO is not well understood.

Let us discuss how polymorphism in the genes coding for transporters causes inter-human variation in the toxic responses using few examples:

1. Humans are exposed to the toxicant methyl-mercury (MeHg) by consuming fishes caught from contaminated water. MeHg is well absorbed from intestine and can cross blood-brain and placental barriers using various transporters. MeHg after being conjugated with glutathione is effluxed by cells of kidneys and liver in urine and bile respectively. MeHg can cause damage to foetal brain in mothers who have high level of toxicant in the blood due to consumption of contaminated fishes. Studies have found that women carrying a particular allele of transporter ABCC1, are likely to have higher MeHg burden in the body, and higher rate of MeHg transfer across placenta that may affect foetal neuronal development.

2. The drug 'Fenoterol' a β_2-adenoreceptor agonist is a bronchodilator and used to treat asthma. This drug also stimulates β_1-adenoreceptor present in the heart, causing cardiovascular toxicity in some patients. The drug is transported by OCT1 (coded by Gene SLC22A1). People with genetic deficiency of OCT1 when treated with fenoterol, achieve higher concentration of the drug in plasma, causing cardiac adverse effects. In another example, a particular allele of OCT1 transporter reduces the effectiveness of antidiabetic drug 'metformin' in some individuals. In these individuals, the drug is not efficiently absorbed in the intestine, and the transport of drug into liver as also affected.

Table 7.2: Various transporters (genes) involved in transport of endogenous molecules across cell membrane.

ATP-Binding Cassette (ABC) Superfamily	Solute Carrier Organic Anion (SLCO) Superfamily	Solute-Linked Carrier (SLC) Superfamily
ABCA1 Cholesterol efflux onto HDL.	*SLCO1A2* Bile salts, organic anions andcations.	*SLC2A2* Facilitated glucose transporter.
ABCB1 Peptides, steroids, bile salts.	SLCO1B1 Bile salts, organic anions.	*SLC16A11* Transport of pyruvate.

ABCC2 Organic anion efflux.	*SLCO1B3* Bilirubin bile acids, coproporphyrin I and III.	*SLC30A10* Manganese transport.
ABCC3 Efflux of organic anions and glutathione conjugates and glucuronide.	SLCO4A1 Taurocholate, T3, prostaglandin.	*SLC17A1* Sodium-dependent phosphate transporter 1, and renal transporter of uric acid.
ABCB11 Bile salt transport.	SLCO4C1 Thyroid hormones.	*SLC8A1* Sodium(Na^+)-calcium(Ca^{2+}) exchanger 1.
ABCB6 Iron transport.	*SLCO1C1* Steroid conjugates and thyroid hormones.	*SLC39A8* Zinc transport.
ABCC4 & ABCC5 Nucleoside transport.	SLCO2A1 Eicosanoids and prostaglandins.	*SLC14A2* Urea transporter.
ABCD1 Very-long-chain fatty acid (VLCFA) transport.	SLC10A6 Bile acid and bile salt.	*SLC12A1* Sodium–potassium–chloride co-transporter.
ABCG1 Cholesterol transport.	SLC22A25 Organic anion transport.	*SLC6A13* GABA transporter.
ABCB8 Peptide trafficking across membranes inside cell.	*SLCO1B7* Bile acid and bile salt transport and sodium-independent organic anion transport.	*SLC6A15* Branched-chain amino acids, particularly leucine, valine, isoleucine, and methionine.
ABCG2 Efflux of Organic anion and sulphate conjugates, and biliary excretion.		*SLC7A9* Transport of cystine and neutral and dibasic amino acids.

7.2.3 Variation in Response due to Polymorphism in Xenobiotic Target Molecules

Several xenobiotics initiate their toxic responses by interacting with body's protein molecules including: membrane and intracellular receptors; membrane transporters and channels; carrier proteins; enzymes and other proteins such as those involved in cytoskeleton (actin, tubulin, etc.). These target protein molecules may be coded by genes which exhibit genetic polymorphism. Thus, existence of more than one alternate form of 'protein target molecules' (coded by each allele of a polymorphic gene) in a population is also responsible for the observed inter-human variation in response to toxicants/drugs.

Let us discuss this with a few suitable examples:

The β1-adrenergic receptor (ADRB) antagonists (such as propranolol, atenolol, metoprolol) are used to treat patients with essential hypertension. Studies have shown that in some individuals, an SNP in ADRB replaces'arginine' at 389th position with 'glycine' which causes increase in response to the drugs.In another example;D3-dopamine receptor agonists (e.g.haloperidol) are used to treat schizophrenia. Patients in whom an SNP replaces 'serine' with 'glycine' at 9th position in D3-dopamine receptor are at risk of developing 'tardive dyskinesia' (repetitive involuntary body movements).

From the above discussions (sections 7.2.2–7.2.4), you may have realized how responses to toxicants anddrugs are affected by the genetic makeup of individuals.

SAQ 1

State whether the following statements are true or false:

a) Genetic variations among individuals of a population, is the only cause for observed inter-human differences in responses to drugs and toxicants (True/False).

b) Existence of more than two alleles for xenobiotic metabolizing enzymes in a population may cause inter-human differences in responses to drugs and toxicants (True/False).

c) SNPs are the most common type of genetic variation in the human genome (True/False).

d) A nucleotide substitution in the gene, which causes replacement of original amino acid in the protein with another is called synonymous substitution (True/False).

7.3 MOLECULAR MARKERS

We have seen under the heading 'genetic polymorphism' (see box above) that– the variation 'or' polymorphism in DNA sequence is alsofound in those regions of the genome which have no known function. Such silent DNA variations (polymorphisms) do not cause any change in the function of organismsor appear as visible phenotype (phenotypically neutral). These polymorphisms however are useful as 'molecular markers', sometimes also referred as 'molecular genetic-markers' or 'DNA-markers'. A molecular marker is a polymorphic DNA-sequence with known physical location (locus) on a chromosome. Molecular markers are used in various fields such as, medicine, plant breeding, phylogenetic analysis, forensic investigations, etc. Since DNA segments in chromosomes located close to each other are inherited together, a molecular marker can thus be used as a tag for genes located in close vicinity, for which other details are not yet known or available. In this way, molecular markers can be used to monitor the inheritance of genes and alleles involved in a disease (useful in disease diagnosis and genetic counselling), or those genes that make persons susceptible to toxicants/ADRs (useful in preventing ADRs in patients with particular genotype).An ideal molecular marker should be highly polymorphic, co-dominant, and evenly distributed in the genome. Table 7.3 shows some of the widely used types of molecular markers.

Table 7.3: A few types of molecular markers.

Types of molecular markers
1. Restriction Fragment Length Polymorphism (RFLP)
2. Randomly amplified polymorphic DNA (RAPD)
3. Microsatellites
4. Amplified fragment length polymorphism (AFLP)
5. Single Nucleotide Polymorphism (SNP)

7.4 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) AND PHARMACOGENETICS

Pharmacogenetics–a sub-discipline of pharmacology, studies effects of various alleles of one 'or' a few genes on inter-human variation in drug response (including ADRs). It is now known that the response to drugs is influenced by several genes (which may be polymorphic), and the observed variation in response is the outcome of complex interactions among the products (metabolic enzymes, receptors, transporters, channels, etc.) of these genes. A more holistic approach–*Pharmacogenomics* identifies influence of all the genes (and their alleles) in the genome that may affect the response to drugs. Pharmacogenomics is a promising approach that can help in identifying the 'right dose' for a person based on his/her 'genetic makeup', in other words, a dose which is effective but free from any ADR. The corresponding sub-discipline in toxicology–*Toxicogenomics* studies influence of all the genes in a genome on the toxic response to a chemical.

Single Nucleotide Polymorphisms (SNPs)are the most common type of polymorphism found in the genome. SNP is the key molecular marker used in defining a person's genetic susceptibility to toxicants, chances to develop a disease and ADRs to drugs (in pharmacogenetic and pharmacogenomics approaches). SNPs located close to a gene (or allele) suspected to make individuals prone to exaggerated response to toxicants and ADRs, can be used as surrogate marker for the genes itself. Many SNPs directly mark a gene if the formerare located in the coding regions. These features make SNPs a preferred molecular marker to draw a high-density molecular marker map of the genome.

SNPs are used as molecular markers in 'Genome Wide-Association Studies' (GWAS). The main aim in GWAS is to identify 'genetic variations' (such as SNPs) in the genome associated with a particular trait/disease/condition (such as extent of response to drugs and toxicants). In GWAS, genomes of several individuals are surveyed to look for SNPs associated with the disease and the results are compared with those of individuals without the disease under concern. Since the GWAS involves whole genome, the study identifies several SNPs that are associated with the disease or condition.To identify SNPs in the genome, SNP-arrays (a chip dotted with arrays of fragments of DNA used as probe) are used. The samples (DNA fragments form genome of individuals) are brought in contact with the chip and observed for hybridization with the probe.

SAQ 2

Fill in the blanks with appropriate words:

a) Scientific approaches in which association SNPs and the disease under concern is studiedare called............. .

b) Sub-discipline of pharmacology where effect of single or a few gene variants is studied is called.......... .

7.5 SUMMARY

- There exists an inter-human variation in response to drugs and toxicants in a population. The most important cause of this variation is genetic polymorphism in the genes coding for xenobiotic metabolizing enzymes, xenobiotic transporters and xenobiotic target sites.

- More than one allele of xenobiotic metabolizing enzymes may exist in the population which may code for the enzyme molecules with reduced or enhanced enzymatic activity. This causes some individuals of the population to metabolize xenobiotics at a slower rate than others. The former group of individuals thus may have a higher burden of xenobiotic in the blood leading to exaggerated toxic response or Adverse Drug Reactions. Similarly, existence of more than one allele for xenobiotic transporters causes some individual to absorb more xenobiotics than others resulting in observed variation in response.

- SNPs are single nucleotide DNA variations at a particular locus in the genome. SNPs are the most commonly used molecular markers in the pharmacogenetics and pharmacogenomics approaches.

7.6 TERMINAL QUESTIONS

1. What are the causes of inter-human variation in responses to xenobiotics in a population?

2. Discuss genetic polymorphism.

7.7 ANSWERS

Self Assessment Questions

1. a) False b) True c) True d) False

2. a) Genome Wide-Association Studies

   b) Pharmacogenetics

Terminal Questions

1. Inter-human variation in response to xenobiotics is caused by two main variables i) genetic polymorphism in the population and ii) environmental factors:age and life stage of individual, gender, co-existing disease, lifestyle, nutritional status, etc. Polymorphism in the genes coding for proteins involved in xenobiotic transport, metabolism and xenobiotic target molecules, accounts for most of the observed inter-human variation in response to toxicants/drugs. Existence of more than one allelic form of these genes causes differences in absorption, metabolism and excretion of xenobiotics, and interaction with the target molecule among different individuals of the population. This results in the variation in toxic response.

2. Genetic polymorphism is the difference in the nucleotide sequence at a particular location (locus) on a chromosome which occurs with a frequency of 1% or higher in a population. The differences or genetic variations at various loci may be in the form of– i) variations in a single nucleotide (Single Nucleotide Variations or SNVs), ii) insertion/deletion of few nucleotides and, iii) variations in the numbers of sets of ‘unique

repeating nucleotide sequence' in a stretch of chromosome (such as Copy Number Variations or CNVs). SNVs are the most common type of genetic variation, widely used as molecular markers in studies aimed to decipher association between molecular markers and a particular disease or a genetic condition.

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