Unit 8

CUTANEOUS AND PULMONARY TOXICITY

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8.1 INTRODUCTION

Many toxicants do not show same level of toxicity in all the organs; and instead, they selectively produce toxicity in one or few organs. The organ/s in which a given toxicant produces toxicity is known as 'target organ' of that toxicant. This organ's selective toxicity is largely due to molecular and biochemical differences among the cells of various organs. The extent of damage by toxicants to various organs is measured by several specific biochemical and molecular tests. These tests have been widely used to monitor exposure and effects of toxicants at population level. In this unit, we will discuss the effects of toxicants on skin and respiratory system.

The vast surface area of skin is exposed to a variety of toxicants. The unique structural and biochemical properties of skin prevent many toxicants from crossing the skin-barrier and entering into the blood to show systemic toxicity. Several toxicants show adverse effects ranging from simple rashes to skin cancers.

Our respiratory system is exposed to several toxic gases, volatile liquids and particulate matter. Though the combined action of mucus and ciliary movement in the respiratory tract lumen can remove coarser particulate matter, fine particles can penetrate to alveoli and are responsible for the disease called‘ Pneumoconiosis’. Toxic gases and volatile liquids can cause mild irritation in nasal passage or upper respiratory tract. Inhalation of a high concentration of these substances can cause damage to the alveolar cells, and lead to conditions which prevent exchange of gases in the lungs.

In the last section of this unit, we will discuss management and treatment of poisoned patients. The method to treat a poisoned patient is to stabilize the vital parameters indicative of respiratory and cardiovascular functions. For successful treatment, it is often useful to find out–the ‘class or type of poison’ and ‘the dose’ to which patient was exposed, and the timing of exposure. Past events ‘or’ patient’s history, physical and clinical evaluations are used to get a clue about the poison and dose of exposure. Techniques such as those which help in the quick removal of unabsorbed poison, and those which are used to reduce concentration of poison in the blood are undertaken in parallel. Use of specific antidotes is of limited use for various reasons including availability of antidotes only for a limited number of poisons.

Expected Learning Outcomes

After studying this unit, you should be able to:

- understand basics of organ specific toxicity;
- learn structure/anatomy of skin, and responses of skin to various toxicants;
- learn structure/anatomy of lungs, and its responses various toxicants;
- learn strategies for the treatment of poisoned patients; and
- know, fundamentals of antidotes including antivenom.

8.2 ORGAN TOXICITY

In the previous units (see Units 1 and 2), we have learnt that many toxicants do not show same degree of toxicity in every organ; they selectively harm one or few organs of the body. These organs are known as ‘target organ/s’ of toxicity. Further, in a given organ, toxicants do not affect all the cell types equally, instead show selectivity for one or few cell types. Thus, we can denote a chemical/substance as being neurotoxicant or hepatotoxicant, or nephrotoxicant, etc. depending on the organ/system where it shows its primary toxic action. Let us discuss the main reasons for organ selective toxicity by toxicants with examples.

A toxicant shows selective toxicity because:

i. It interacts with a specific target molecule found only in the cells of the target organ /system. For example, the sedative barbiturates show their main toxic action (in case of overdosing) on CNS because they bind and enhance activation of the GABA–chloride receptor (found in CNS),and causecoma and death.

ii. It inhibits certain process in cells of the target organ/system, which is more crucial for the survival or functioning of the cells of target organ than other cells of the body. For example, cyanide inhibits aerobic respiration in cells. Cells of CNS and heart are most sensitive to cyanide toxicity because of a high demand of energy and a dependence on aerobic respiration to obtain energy.

iii. It is selectively taken up by the cells of target organ/system. For example, microcystins are hepatotoxic because they are selectively taken up by the hepatocytes via membrane transport proteins.

iv. It can be bio-activated in the target organ. For example, hepatocytes, lungs and kidneys are susceptible to CCl_4 toxicity because it is extensively activated to a toxic metabolite in these organs.

v. It can affect several organs but the toxic effect becomes apparent in those organs which are not able to repair or adapt to toxic insult. For example, neurons of CNS and cardiac muscles have no or limited capacity to repair, therefore, are unable to replace cells that die due to toxic insult.

In this unit, we will discuss about skin and lungs as target organs for certain toxicants. We will learn about the structural features of skin and lungs, and pathological changes caused by toxicants. In Units 9–11, we will learn about effects of toxicants on various other organs including liver, kidneys, heart, etc.

8.3 CUTANEOUS TOXICITY

Skin, the largest organ, covers almost the entire body from outside. It acts as body's first protective barrier against microbes, physical agents and chemicals. Skin also performs several important physiological functions e.g. thermoregulation, water and electrolyte balance, synthesis of vitamins, sensation, and immunity. The vast surface area of skin (about 1.5–2 m^2) is exposed to varieties of chemicals (such as cosmetics, solvents, pesticides, water pollutants, etc.). These chemicals can be absorbed via skin and enter into the blood, and show systemic toxicity or can adversely affect skin at the site of exposure. People working without protective gears in various industries, farms, etc. are vulnerable to skin toxicity by chemicals they handle. In general population, excessive application of cosmetic products, household cleaners, and solvents are the major source of skin toxicants.

Let us first discuss the structure of skin, and after that, learn the various pathological changes caused by toxicants to skin.

8.3.1 Structure of Skin

We have already discussed about the structure of skin in Unit 5. Let us recall a few features of skin which are important from toxicological point of view. Refer to the Fig. 8.1, skin is made of three parts i.e. epidermis, dermis and hypodermis. The outer most layer of *epidermis*–the 'stratum corneum' is made of sheets of a nucleated flat cells, which are rich in fibrous protein keratin (Figure 1B). The cells are embedded in a lipid rich matrix, thus this layer of epidermis acts as a barrier to the hydrophilic substances. The cells of innermost layer of epidermis 'stratum basale' continuously divide and provide new cells to the epidermis. Epidermis also contains other cells such as 'melanocytes' which give skin its characteristic colour and protection from ultraviolet rays. The 'Langerhans cells' found in the epidermis are cells of the immune system. These cells are involved in triggering immune response in skin against toxicants. Epidermal cells contain various xenobiotic metabolizing enzymes (such as CYPs, FMOs, dehydrogenases, etc.). The bio-activation of xenobiotics by these enzymes in skin may result in sensitization reactions, carcinogenesis and other toxicities. The *dermis* is a highly vascular layer and is composed of collagen and elastic fibres. Dermis also houses 'hair follicles', 'sweat glands' and' sebaceous glands' (collectively called 'dermal appendages'), and sensory neurons. Water-soluble substances which otherwise cannot penetrate epidermis due to high lipid content in stratum corneum, can enter skin via dermal appendages and reach dermis, from where it can be absorbed into blood to produce systemic toxicity.

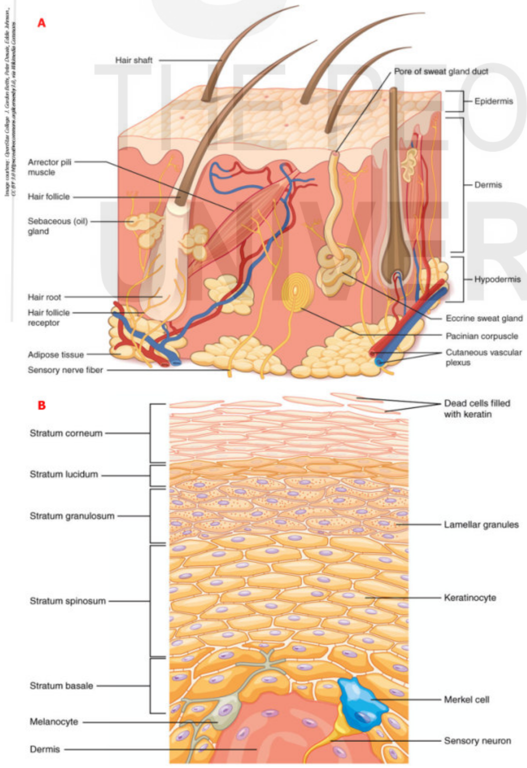

Fig. 8.1: Anatomy of skin, (A) gross structure (B) various cellular layers in epidermis. Source: OpenStax College J. Gordon Betts, Peter Desaix, Eddie Johnson., CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons and OpenStax College J. Gordon Betts, Peter Desaix, Eddie Johnson., CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons.

8.3.2 Responses of Skin to Toxicants

Toxicants can show varieties of adverse effects in skin, and dermal appendages. The effect of toxicants may manifest in the form of skin irritation, rashes, blisters and corrosion (contact dermatitis), acne, skin depigmentation and cancer. There are three basic mechanisms by which toxicants or their metabolites can harm skin *viz.i)* by direct damage to skin (such as irritation, corrosion, genotoxicity), *ii)* by phototoxic reactions, *iii)* by immune-mediated effects (such as allergies).

Let us see a few types of toxicant-induced injury to skin.

Contact dermatitis

It is a condition in which skin gets rashes, blisters, or is inflamed due to direct contact with chemicals. Contact dermatitis is of two types:

i) Irritant contact dermatitis *ii)*Allergic contact dermatitis

Irritant contact dermatitis is a more common toxicant induced skin injury than allergic contact dermatitis. It is due to the contact of skin to a toxicant for a prolonged period ora single event of contact with a high concentration of a toxicant. Skin burns due to strong acids, alkalis and other corrosive chemicals is a type of irritant dermatitis called ‘corrosion’. In this case, contact of skin with toxicants immediately cause death of the cells and severe damage to the tissue. The most common form of irritant dermatitis is ‘chronic cumulative Irritant dermatitis’ which is due to repeated (chronic) exposure to mild irritants such as soaps, solvents, detergents, etc.

Allergic contact dermatitis is a delayed T-cell mediated hypersensitivity response in which the toxicant penetrates the epidermis during its first contact with the skin, and binds covalently to proteins in the skin. The protein-toxicant complex is processed by Langerhans cells and presented to helper-T cells. During this initial phase (1-3 weeks duration), T-cells are sensitized by antigen and expand in numbers. On subsequent exposure to the same toxicant, a more vigorous and quicker response is elicited in which the pre-sensitized T-cells secrete several signalling molecules. This causes recruitment and activationof macrophagesand subsequent damage to the surrounding skin tissue. Hundreds of chemicals found in consumer products and industrial chemicals (including drugs, antiseptics, preservatives, fragrances, glue material, metals, etc.) are known to cause allergic contact dermatitis.

Photodermatoses

In this type of adverse reaction, simultaneous exposure of skin to photo reactive chemicals and ultraviolet spectrum (UV)of sunlight causes skin injury (such as reddening, rashes, blistering).Depending on the mechanisms involved in skin damage, photodermatosescan be divided into two types *viz.*‘phototoxic dermatitis’ and ‘photoallergic dermatitis’. In the first type, the photoreactive toxicant/chemical (such as furocoumarins, anthraquinone dyes, drugs such as tetracyclines, sulfonamides, etc.) absorbingin UV spectrum of sunlight is transformed into excited or high energy states. While returning to the ground state, these toxicants transfer electrons to the oxygen producing reactive oxygen species which can damage cells in the skin. In the second type i.e. photoallergic dermatitis', the toxicants excited by the UV spectrum of sunlight, react with endogenous macromolecules such as proteins, and the complex (protein-toxicant complex) is processed by Langerhans cells to sensitize T-helper cells. This produces delayed type of hypersensitivity response (see above under contact dermatitis). A few chemicals that can cause photoallergic dermatitis include drugs (ketoprofen, phenothiazines, etc.); components of sunscreen (benzophenones);fragrances (musk ambrette);and halogenated salicylanilides.

Cutaneous carcinogenesis

Toxicants can cause skin cancer. Exposure to soot (such as during cleaning of chimney) is associated with scrotal skin cancer. Other similar chemicals such as coal tar, creosote oils rich in polycyclic aromatic hydrocarbons (PAH) also cause skin cancer. PAHs are converted to reactive metabolites by CYPs located in epithelial cells of skin and hair follicles. These reactive metabolites can react with DNA and cause carcinogenesis. Arsenic is known to cause squamous cell carcinoma and basal cell carcinoma by mechanisms including chromosome aberrations, and by generation of reactive oxygen species. Chronic irritation of skin, inflammation and chronic exposure to UV radiation increases risk of skin cancer by toxicants. For example, prolonged use of psoralens for treatment of psoriasis and concurrent exposure to UV increases risk of squamous cell carcinoma.

SAQ 1

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

i) The epidermal cells of skin contain xenobiotic metabolizing enzymes which can increase toxic potential of toxicants (True/False).

ii) Langerhans cells in skin are involved in allergic reactions (True/False).

iii) In photodermatoses, a toxicant requires activation by UV light in order to show toxic effects (True/False).

iv) A toxicant can cause allergic contact dermatitis immediately after making contact with the skin (True/False).

b) Fill in the blanks with appropriate words:

i) The type of irritant dermatitis caused by excessive use of soaps and detergents is...........

ii) The 'hair-follicles', 'sweat glands', and 'sebaceous glands' are collectively denoted as ....

8.4 PULMONARY TOXICITY

The respiratory system is exposed to several air borne toxicants such as vapours of volatile solvents, gases and particulate matters. Upon contact with the respiratory tract, these inhaled toxicants can harm tissues in nasal passage, trachea, bronchioles and alveoli. The inhaled toxicants may also be absorbed in the blood and carried to distant organs and show toxic effects. In addition to inhaled toxicants, respiratory system is exposed to toxicants absorbed via other routes (such as gastrointestinal tract) and brought to lungs via blood. In this section, we will discuss about toxicants which affect respiratory system, particularly lungs.

8.4.1 The Structure of Respiratory Tract

The respiratory tract can be divided into two functional parts: i) conducting system, and ii) gaseous exchange system. The *conducting system* is composed of nasal cavity, pharynx, trachea, bronchi, and bronchioles (Fig. 8.2a). The functions of this part of respiratory tract iscleansing, warming and moistening of inhaled air and to provide a pathway through which air moves and is delivered to alveoli for gaseous exchange. The *gaseous exchange system* is composed of respiratory bronchioles, alveolar ducts, alveolar sacs and blind ending alveoli (Fig. 8.2a). The main region of lungs involved in gaseous exchange is 'alveoli', the thin walls of which have an extensive network of capillaries (Fig. 8.2c). It is at this interface (called 'respiratory membrane' i.e. walls of alveoli and capillary) that the gases filling the alveolar sac, cross the flat thin cells of alveoli and the endothelium, and reach the blood (Fig. 8.2c).

The conducting system is lined with epithelium composed of tall cells of two main types: the 'mucus secreting' cells(ciliated pseudostratified columnar epithelium) and 'ciliated cells'(having numerous cilia at their cell surface).Thick mucus lining the conducting system traps particles inhaled with air. Mucus with trapped particulate matter is regularly propelled by the movement of cilia and removed from the respiratory tract. This action helps cleansing the inhaled air. The final branch of conducting system is 'terminal bronchioles' which open in reparatory bronchioles (Fig.8.2b). The terminal bronchioles and respiratory bronchioles are lined with a small number of cells called 'Clara' or 'Club cells'. These cells act as stem cells, and synthesize substances that act as surfactants. The surfactants prevent alveoli from collapsing due to the surface tension.

The alveoli of gaseous exchange system are lined with two types of cells i.e. type Ipneumocytes (flat cells covering most of the lung surface) and type II pneumocytes. The latter cell type areround in shape and produce surfactants. These cells can also differentiate into type I cells in case of lung damage.

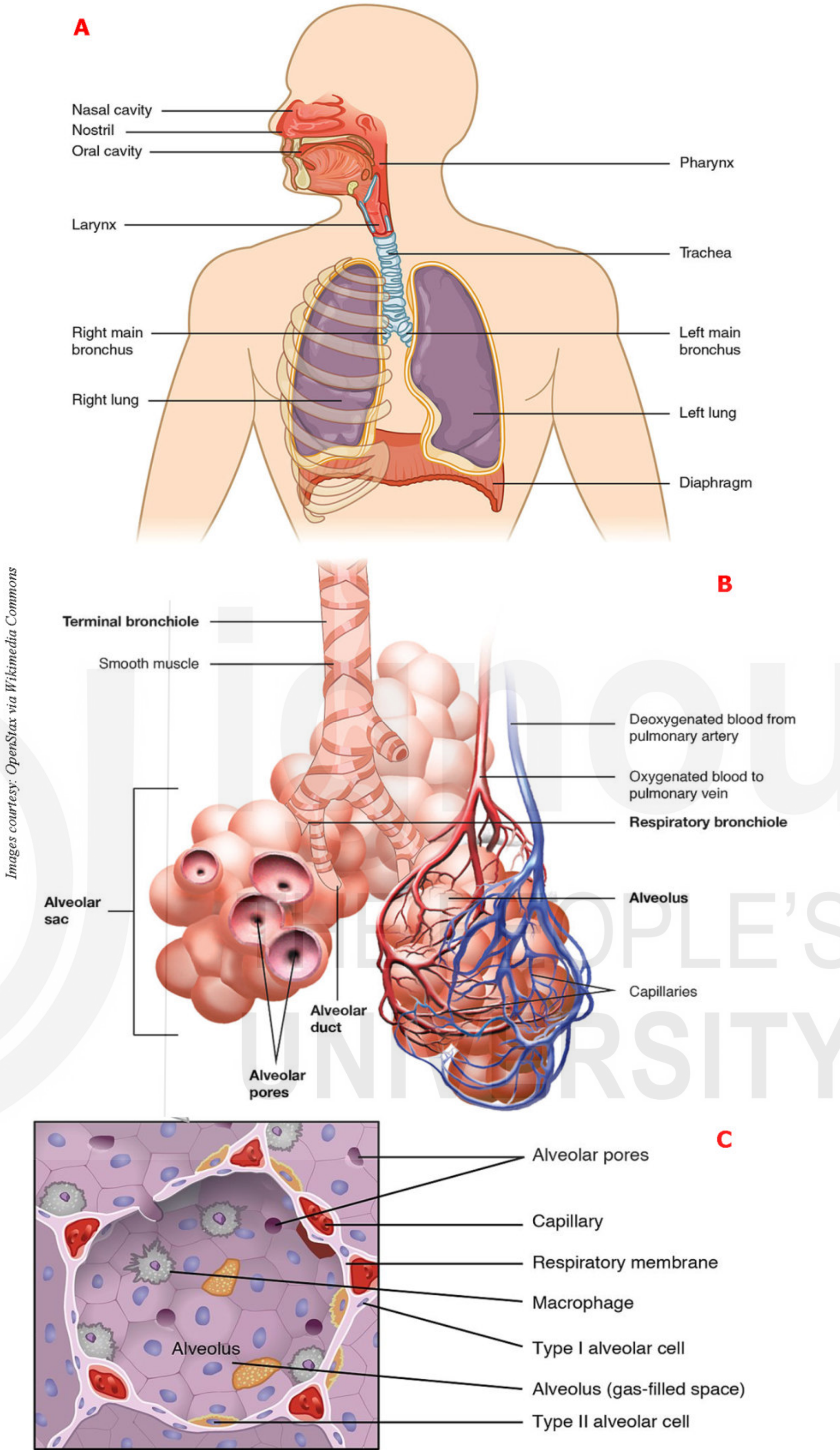

Fig. 8.2: Schematic of respiratory system; (A) the conducting system, (B) the gaseous exchange system, and (c) microstructure of alveoli. Source: a) OpenStax College, CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons b) OpenStax College, CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons c) OpenStax Anatomy and PhysiologyOpenStax, CC BY 4.0 httpscreativecommons.orglicensesby4.0, via Wikimedia Commons.

8.4.2 Responses of Respiratory Tract to Toxic Insult

The toxic responses of respiratory tract may range from irritation in nasal passage, bronchospasm, to severe conditions such as enema and cancer. Let us see a few main toxic responses of respiratory system with an emphasis on damage to lungs.

Irritation, bronchoconstriction and pulmonary edema

Irritation of respiratory tract is the most common response to chemicals such as chlorine, ammonia, etc. Short term exposure to such chemicals induces secretion of mucus in the nasal cavity. Irritant substances reaching further to the lower part of respiratory tract can cause broncho constriction due to contraction of smooth muscles and is accompanied by accumulation of thick mucus in the airways. This condition makes breathing difficult. Continued or excessive exposure to irritants and corrosive chemicals (ammonia, HCl, NO_2) can cause necrosis of the alveolar cells, increase in permeability of epithelial lining, followed by pulmonary edema that may be fatal.

Fig. 8.3: Section through lungs, showing deposition of coal dust together with fibres in a coal worker's lung.Image courtesy: *Yale Rosen from USA, via Wikimedia Commons Source:Yale Rosen from USA, CC BY-SA 2.0 httpscreativecommons .orglicensesby-sa2.0, via Wikimedia Commons*

Chronic Obstructive Pulmonary Disease (COPD)

These are the group of diseases in which flow of air in the reparatory tract is blocked resulting in difficulty to breathe. Chronic bronchitis and emphysema and are the most common COPD. In chronic bronchitis, there is a long-term inflammation in airways and excessive secretion of mucus. Cigarette smoke, repeated exposure to ammonia, sulphur dioxide, and occupational exposure to carbamates and organochlorine pesticides, etc. are known to cause chronic bronchitis. Emphysema is permanent enlargement of air space (alveolar duct and alveolar sac) and destruction of their walls. Cigarette smoking, inhalation of toxic gases such as sulphur dioxide, and particulate matter can cause emphysema.

Pulmonary Fibrosis

In pulmonary fibrosis, there is deposition of collagen fibres in the walls of alveoli which cause impairment in gas exchange. Fibrosis in large part of lungs can result in respiratory failure and death. Inhalation of dust of certain substances such as silica, carbon, asbestos, etc. can induce fibrosis in lungs and cause the disease called 'Pneumoconiosis'. In this disease, fine particles that are not cleared in upper respiratory tract reach deeper in the respiratory tract and get deposited in the alveolar region. The particles are taken up by alveolar macrophages. The activated macrophages and other immune cells release chemical mediators that finally activate fibroblasts to deposit fibres around the particles. The black nodules of fibres and coal dust can be seen in the lungs of coal workers suffering from' Coal workers' pneumoconiosis' or 'Black lung disease'(Fig. 8.3).

Lung cancer:

Currently over 70% cases of lung cancer are caused by cigarette smoking. Several other environmental chemicals such as beryllium, arsenic, cadmium, chromium, nickel, asbestos, etc. also promote lung cancer. Carcinogens such as benzopyrene (a component of cigarette smoke)are known to damage DNA, and asbestos causes activation of macrophages that initiate DNA damage through free radicals and development of cancer.

8.4.3 Systemic Lung Toxicants

There are numbers of toxicants that enter blood via other routes and reach lungs through pulmonary circulation. These toxicants can cause severe lung injury. Let us see examples of a few such toxicants.

Bleomycin

This chemotherapeutic agent that can cause injury to type I pneumocytes and endothelial cells, leading to edema and haemorrhage than may progress to fibrosis. Bleomycin is known to make a complex with iron and promote generation of reactive oxygen species inside the cells and cause damage to DNA.

Cyclophosphamide

This immunosuppressant drug causes pulmonary fibrosis. It is metabolized by CYP 450 to the toxic metabolite acrolein in lungs which can initiate lipid peroxidation and damage to alveolar cells.

Paraquat

Lungs are the main target organ of this herbicide. After its entry into the blood (for example via gastrointestinal tract), paraquatreaches lungs and is taken up by type I and type II pneumocytes. Inside the cells, paraquat cause generation of free radicals by redoxcycling that causes cell death and fibrosis.

Busulfan

This anticancer drug causes damage to pulmonary epithelial cells, particularly type II cellsin some patients. The toxic injury or complications such as alveolar damage begin to appear within 8-10 months of treatment. The patients develop progressive respiratory failure and eventually death.

SAQ 2

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

i) The alveoli are lined with pseudostratified epithelium (True/False).

ii) In emphysema, there is enlargement of lung space which increases the capacity of lungs to absorb oxygen (True/False).

iii) Mucus secretion in respiratory tract helps in trapping and removal of particulate matter (True/False).

iv) Clara cells found in the nasal cavity are responsible for secretion of surfactants (True/False).

b) Fill in the blanks with appropriate words:

i) Inhalation of dust particles may cause....................

ii) The cells that line most of the surface area of alveoli is................

iii) ‘Black lung disease’ is caused due to the deposition of................. in lungs.

8.5 TESTS FOR EVALUATION OF TOXICITIES IN DIFFERENT ORGANS

Different organs of the body are specialized to perform certain physiological functions. The cells of various organs have two major types of biochemical and molecular processes: those which are necessary for survival of the cells, and others which enable cells of organs to perform the specialized physiological functions. For example, biochemical pathways (and thus biomolecules such as enzymes, proteins and metabolites) responsible for the production of energy in the cells are similar in all cell types of the body, whether they belong to the liver, heart or nervous system. These cells, in addition, contain biomolecules responsible for the: generation of impulse and muscle contraction (e.g. heart cells), catabolism or synthesis of metabolites (e.g. liver cells), and generation and propagation of nerve impulse and synthesis of neurotransmitters (e.g. cells of nervous system). Toxicant-damaging or killing cells of an organ may result in the release of specific biomolecules in the blood or affect the physiological functions which can be measured or tested. These measurements therefore can be used to monitor toxicant-induced organ damage and are called 'Biomarkers', which can be defined as measurable indicators or signs of organ damage or organ malfunction. Thus, appearance, increase or decreases of biomolecules (proteins, enzymes, metabolites, etc.) in the blood and urine samples, or alteration in physiological parameters indicative of toxic damage to organs are 'biomarkers'. For example, increase in Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in blood are routinely used as biomarkers for liver damage; Electrocardiogram (ECG),creatine kinase,cardiac troponin are used for monitoring cardiac malfunction or toxicityand; Glomerular filtration rate (GFR), presence of proteins and enzymes in urine are used to assess renal toxicity.

8.6 GENERAL MEASURES AND TREATMENT OF POISONING CASES

In this section, we will briefly discuss the interventions and methods used by clinicians to manage cases of poisoning. To treat a poisoned patient brought to the hospital, a step wise approach is followed by the doctors:

1. Stabilization or control of vital parameters of the patient,
2. Clinical evaluation (history, toxidromes, laboratory tests, radiographic examination)
3. Decontamination
4. Elimination
5. Antidotes administration
6. Monitoring and clinical follow up

The first step is to assess the condition of patient and stabilize or correct the vital parameters including Airways, Breathing and Circulation or ABC. In this step, the patient is evaluated for the ability to inhale and exhale properly, obstruction in respiratory tract, adequate oxygen level in the blood or haemoglobin oxygen saturation, pulse rate, blood pressure, cardiac arrhythmias, etc. If needed, patient is provided with ventilator support and emergency life-saving medicines to correct the vital parameters. Once the patient is stabilized, other steps (the early steps such as 2 and 3 in particular) are initiated in parallel.

The purpose of *clinical evaluation* is to arrive at the class of poison, amount of poison, and time of exposure to the poison when a patient is brought to the hospital. In cases where patient is unconscious, uncooperative or not willing to tell the doctor about the amount and timing of poison taken, it is necessary to gather information about the patient from all possible sources. This may include gathering history (by interviewing, relatives, friends, etc.) as: where patient was found, what was mental status of patient, when last seen, how many pills from pouch or wrapper are missing, suicide note, past medical history or previous suicide attempts, etc. This approach may provide crucial leads to doctors about the level and timingof exposure to poison, and direct the next course of action.

In combination with history, examination of patient's clinical presentation such as blood pressure, pulse, temperature, size of pupil, might also give clues about the class of poison to which the patient was exposed. For example, symptoms such as: profuse salivation, tears and sweating, miosis, reduced pulse rate, congestion in airways are indicative of over activation of cholinergic system. In this case,it is expected that patient was exposed to poison such as organophosphates, carbamates, toxic mushrooms, etc. which activate cholinergic system of the body. A collection of clinical signs and symptoms which indicate that a patient was exposed to a particular group of chemicals is called 'toxidrome' or 'toxic syndrome'. There are toxidromes for poisoning due to: opioids, sedative-hypnotic drugs, sympathomimetic, anticholinergic substances, etc.

Although history of patients and toxidromes are useful to doctors, patients may not present all the symptoms that fit in a particular toxidrome, and the history may not be reliable. Therefore, additional measures are taken such as performing clinical diagnostic tests: blood count, serum electrolyte, BUN, creatinine, liver function test, anion-gap, osmol-gap, electrocardiogram, radiographic examination, etc. These routine tests may indicate as to which group of poison and the amount to which patient was exposed. An elevated anion gap with acidosis is associated with poisoning due to ethanol, methanol, ethylene glycol, salicylate, iron, etc.

In cases where not much time has elapsed since the patient was exposed to the poison, steps are taken to remove poison (or decontamination) from the site of exposure. This prevents further absorption of toxicant intothe systemic circulation. For example, if exposure to a toxicant is via skin, the clothing contaminated with the poison must be immediately removed and skin washed thoroughly avoiding skin abrasions. In cases of exposure through inhalation, patient should be immediately removed from the toxic environment. However, in the case of exposure via oral route, several interventions are done in order to remove poisons:

i. Emesis

ii. Gastric lavage

iii. Activated charcoal

iv. Whole bowel irrigation

In *emesis*, the patient is given substances which induce vomiting (such as syrup of ipecac). In *gastric lavage,* a tube is passed into the stomach of the patient via mouth and small quantity of warm saline is introduced. The content of stomach is then aspirated. The procedure is repeated till no particulate matter is seen in the aspirate. This procedure removes any unabsorbed pills,or fluid from stomach. To be effective, gastric lavage is to be performed within 1-2 hours of the ingestion of poison. Gastric lavage is contraindicated in patients with cases of recent surgeries, pregnancy, ingestion of acids, patients with compromised airways and susceptibility to gastrointestinal bleeding. Introduction of slurry of *activated charcoal*in the stomach by voluntary intake or through orogastric tube is an efficient method to remove poisons from stomach. The large surface area of particles of activated charcoal has the property to adsorb organic compound, thus toxicants such as: many drugs and natural poisons can be removed by using this procedure. The procedure *whole bowel irrigation* can be used even after several hours have lapsed since the ingestion of poison. In this method, a large volume of fluid containing non absorbable substance such as polyethylene glycol is introduced into the stomach over a period of 2-6 hours. The ingested poison in this procedure is expelled via rectal route together with introduced fluid.

In cases where poison has already got absorbed into the systemic circulation in a significant amount, there are certain procedures which are used for rapid removal of poison from blood:

i. Urine alkalinisation

ii. Hemodialysis

iii. Hemoperfusion

iv. Plasmapheresis

v. Multiple-dose activated charcoal (MDAC)

In *urinary alkalinisation*, the patient is administered sodium bicarbonate solution which makes blood and urine slightly alkaline. In alkaline conditions, weakly acidic toxicants (such as phenobarbitone, salicylates) are in fully ionized state and can be removed efficiently via kidneys (see Unit 5).The technique *hemodialysis* relieson the principle of passage of patient's blood through a semi-permeable dialysis membrane. Toxicants which are of low molecular weight and unbound to the plasma protein pass through the dialysis membrane into the dialysate. The blood now free of toxicant is returned to the body of patient via a second tube. The technique *hemoperfusion* is similar tohemodialysis except that blood is pumped in a cartridge where it comes in direct contact with the activated charcoal. With this procedure, toxicants with higher molecular weight, fat soluble toxicants and those bound to plasma protein can also be removed from the blood, which are otherwise not efficiently removed by hemodialysis. In *plasmapheresis*, the cellular component of blood is separated and resuspended in fresh plasma, and then reintroduced in the patient's body. Though plasma pheresis is an effective method, there is a loss of patient's plasma proteins; and the method cannot be used in patients with bleeding disorders, infections, problems of thrombosis, pulmonary embolism, etc.

In *multiple-dose activated charcoal (MDAC),*oral administration of slurry of activated charcoal is given to the patient every 2-4 hours. This procedure is based on the principle, that toxicants are passively secreted from the walls of intestine into its lumen, which can be adsorbed to the charcoal and removed from the body. MDAC is useful in removing toxicants which undergo enterohepatic recycling.

Administration of specific antidotes is limited because of availability of effective antidotes for only a small number of poisons, and toxic effects of antidotes themselves. In some cases however, administration of antidotes can be life-saving. Antidotes can act via several mechanisms such as, by antagonizing the actions of toxicants at the target molecule, preventing bio-activation of toxicants, enhancing detoxification, and reacting with toxicants to make inert complexes. Table 8.1, provides names of a few antidotes and their main mechanism of action.

Table 8.1: A few antidotes and their mechanism of action

Antidote	**Toxicants against which antidote is given**	**Mechanism of antidote action**
Dimercaprol	metal	chelation of metals
Immunotherapy using antibodies	digoxin	binding of antibodies to poison
Naloxone	opioid	opioid receptor antagonism
Atropine	organophosphate	antagonizes action at muscarinic acetylcholine receptors
Thiosulfate	cyanide	accelerates detoxification of cyanide to relatively nontoxic thiocyanate
Acetylcysteine	paracetamol	detoxifies toxic metabolites of paracetamol
Fomepazole	methanol and ethylene glycol	competitive inhibitor of enzyme alcohol dehydrogenase, which converts methanol and ethylene glycol to their toxic metabolites

After the successful completion of initialphase of treatment, patients at risk of developing complications in the later phase, in cases of delayed toxicity by certain toxicants, or patients with other illnesses, require continued treatment and follow ups. Further, patients of intentional poisoning require psychiatric assessment and constant observation.

8.7 ANTIVENOM

These are the purified antibodies (or crude serum containing antibodies) used for the treatment of venomous bite by animals such as snakes, spiders, scorpion, etc. The antivenom is injected in the body of victims.Antibodies in the antivenom bind and neutralize the components of venom. Idealantivenom should have high potency to neutralize venom components, wide volume of distribution in the body, long half-life in the blood, and free of any adverse reaction in the patient. Antivenom is produced by injecting a small amount of detoxifiedvenom componentsin an animal (such as horse, sheep, rabbit, etc.) for a prolongedperiod of time. The immune system of animals recognizes venom components as foreign material and generates antibodies against it. Once the desired amounts of antibodies are produced, blood is withdrawn from the animals and serum is isolated. This crude serum may be injected into the victims or patients. However, crude serum also contains several blood proteins of the animal in which antibody was produced, and it causes adverse reactions in the patients. In an improved version, crude serum is processed through purification protocols and purified antibody preparation is obtained. The purified antivenom injected in patients produces fewer adverse reactions.

Antivenom produced against venom of one species of venomous animals usually neutralizes venom components of other closely related species. Thus, antivenom produced against venom of one species of snake may be given to patients bitten by other closely related species of snakes.

SAQ 3

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

i) The first step in the treatment of a poisoned patient is the administration of specific antidote (True/False).

ii) In hemoperfusion, blood is dialyzed using a semipermeable membrane to remove toxicant (True/False).

iii) High molecular weight toxicants bound to plasma proteins can be efficiently removed by hemodialysis (True/False).

iv) Use of activated charcoal is one of the efficient methods to remove unabsorbed toxicants from gastrointestinal tract (True/False).

b) Fill in the blanks with appropriate words:

i) A group of symptoms which give a clue about the class of poison involved in poisoning is called....................

ii) The process in which whole plasma of poisoned patient is replaced with the freshplasma and reintroduced in the patient's body is called..................

8.8 SUMMARY

- Many toxicants selectively harm one or few organs of the body. These organs are known as 'target organ/s' of toxicity. Also, in a given organ, toxicants show selectivity for one or few cell types. The selectivity can be due to: presence of specific target molecule in the target organ or certain crucial process which are active only in target organs; are inhibited by toxicants or selective uptake and activation of toxicant by target cells. Injury or damage to organs can be monitored by biochemical and molecular tests, which measure metabolites, enzymes, proteins, etc. released by injured cells in the blood.

- Skin is the largest organ of the body and has a vast surface area. It is exposed to varieties of toxicants. Under normal circumstances, many of these toxicants are not able to cross skin, and thus, are unable to enter systemic circulation. Thus, skin functions as a barrier to toxicants and protects the body. Skin is made of three layers viz. epidermis, dermis and hypodermis. The outer most layer of *epidermis*–the 'stratum corneum' is rich in lipids and thus, acts as a barrier to hydrophilic substances. Skin also has cells that impart complexion, and cells involved in immune system (Langerhans cells). The latter cells are involved in eliciting allergic response of skin against certain toxicants.

- The main pathological changes caused by toxicants in skin are: contact dermatitis, photodermatoses and cutaneous cancer. In contact dermatitis,skin becomes rashy, blistery, or inflamed due to direct contact with chemicals. Certain corrosive and highly oxidizing chemicals at higher concentration can produce acute responses such as skin corrosion and burns wherein cells of skin are immediately killed by the chemicals. Prolonged repeated use of excessive soaps and detergents causes 'chronic cumulative Irritant dermatitis'. In photodermatoses, photoreactive chemicals activated by ultraviolet light (in sunlight) react with cellular macromolecules and cause skin injury. Allergic dermatitis (contact allergic dermatitis and photoallergic dermatitis) is a delayed type T-cell mediated hypersensitivity response in which initial sensitization of skin by toxicants shows exaggerated response on subsequent exposures. Unlike non-allergic responses, allergic responses take weeks to manifest. Certain chemical classes such as PAH, heavy metals (arsenic), etc. are known to cause skin cancer due to their DNA damaging properties by free radical formation mechanism.

- Toxic substances causing injury to lungs are called pulmonary toxicants. Toxic gases, volatile solvents and particulate matter can penetrate respiratory tract and reach alveoli. The toxicants can damage alveolar cells in the lungs and lead to pulmonary edema and fibrosis. These conditions reduce the capacity of lungs to exchange oxygen and carbon dioxide. Substances such as silica, carbon, asbestos, etc. can induce fibrosis in lungs and cause 'Pneumoconiosis'.In this disease, fine particles of these substancesreach deeper in the respiratory tract and deposit in the alveolar region.The particles are taken up by alveolar macrophages. Activation of macrophages as well as other immune cells finally activates fibroblasts to deposit fibres around the particles.

- Poisoned patients brought to the hospitals are treated in a stepwise manner in which first step is to stabilize the vital parameters i.e. respiratory and cardiovascular functions. In cases where patients are unconscious or non responsive and no information is available on the poison to which patient was exposed, the dose and the time post-exposure, doctors rely on interviewing friends, relatives of patient to know the history. The history, physical and clinical evaluation of patient may provide important insights on the poison and dose of poison to which the patient was exposed and help in correct orientation to the process of treatment. Techniques such as induction of emesis, introduction of charcoal in the stomach and gastric lavage are used to quickly remove unabsorbed poison from the stomach. Several techniques are used to remove poison from the blood, such as urine alkalinisation, hemodialysis, hemoperfusion, plasmapheresis and multiple-dose activated charcoal (MDAC). Use of specific antidotes is of limiteduse because of availability of antidotes only for a limited number of poisons. Antivenom is the preparation which is injected in the patients bitten by the venomous animals. The antibodies present in the antivenom neutralize the components of venom and make them ineffective.

8.9 TERMINAL QUESTIONS

1. Why certain toxicants show organ specific toxicity. Discuss briefly.
2. Discuss about contact dermatitis.
3. Mention two systemic pulmonary toxicants and their mechanism of toxicity.
4. Mention interventions undertaken to hasten removal of toxicants from the blood.

8.10 ANSWERS

Self-Assessment Question

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

   b) i) Chronic cumulative irritant dermatitis

   ii) Dermal appendages

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

   b) i) Pneumoconiosis

   ii) Type I pneumocytes

   iii) Coal dust or coal particles

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

   b) i) Toxidrome ii) Plasmapheresis

Terminal Questions

1. Certain toxicants show organ selective toxicity because: *i)* they interact with a specific target molecule found only in the cells of the target organ /system or *ii)* inhibit certain process in cells of the target organ/system which is more crucial for the survival or functioning of cells of the target organ than other cells of the body, *iii)* they are selectively taken up by the cells of target organ/system, iv) they can be bio-activated in the target organ, and v) they can affect several organs but the toxic effect becomes apparent in those organs which are not able to repair or adapt to toxic insult.

2. It is a condition in which skin becomes rashy, blistery, or inflamed due to direct contact with chemicals. There are two types of contact dermatitis viz. *i)* Irritant contact dermatitis *ii)*Allergic contact dermatitis. Irritant contact dermatitis is the common form which is due to the contact of skin toxicant for a prolonged period 'or' a single event of contact to a high concentration of toxicant. Skin burns due to strong acids, alkalis and other corrosive chemicals is a type of irritant dermatitis called 'corrosion' in whichupon contact with the toxicant, there is immediate death of the cells and severe damage to the tissue. The most common form of irritant dermatitis is 'chronic cumulative Irritant dermatitis' which is due to repeated (chronic) exposure to mild irritants such as soaps, solvents, detergents, etc.Allergic contact dermatitis is a delayed T-cell mediated hypersensitivity response. In this type of response, first contact of toxicant with the skin causes sensitization. During this initial phase, T-cells are sensitized by antigen and expand in numbers. On subsequent exposure to the same toxicant, a more vigorous and quicker response is elicited in which the pre-sensitized T-cells begin to secrete several signalling molecules. This causes recruitmentand activation of macrophages and subsequent damage to the surrounding skin tissue.

3. **i)Bleomycin:** It is a chemotherapeutic agent which causes injury to type Ipneumocytes and endothelial cells, leading to edema and haemorrhage that may progress to fibrosis. Bleomycin is known to make a complex with iron and induces generation of reactive oxygen species and damage to DNA. **ii) Paraquat:** upon absorption form gastrointestinal tract,it is taken up by type I and type II pneumocytes. Inside the cells,paraquat causes generation of free radical by redox cycling that causes cell death and fibrosis.

4. The following techniques are used for the rapid removal of poison from blood:

   i. Urine alkalinisation

   ii. Hemodialysis

   iii. Hemoperfusion

   iv. Plasmapheresis

   v. Multiple-dose activated charcoal (MDAC)

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Unit 9

HEPATOTOXICITY

Structure

9.1 Introduction

Expected Learning Outcomes

9.2 Functions and Susceptibility of Liver to Toxic Insult

Functional Anatomy of Liver

Liver Zonation

9.3 Types of liver injuries

Necrosis

Fatty liver (Steatosis)

Cholestasis

Fibrosis and Cirrhosis

9.4 Hepatotoxic agents

Microcystins

Amatoxins

Carbon tetrachloride

Paracetamol

Ethanol

Allylalcohol

9.5 Summary

9.6. Terminal questions

9.7 Answers

9.8 Further Readings

9.1 INTRODUCTION

Liver is metabolically the most active organ of thebody; the cells of liver are involved in varieties of functions which are crucial for survival. Liver is susceptible to toxic insult as it receives a high concentration of absorbed toxicants and for being the primary site for metabolism of xenobiotics. Though liver has capacity to regenerate, extensive or chronic exposure to toxicants can cause serious liver injuries. Many liver toxicants damage the essential ordered microstructure of liver,essential for performing important metabolic functions. In this unit, we will see how certain toxicants interact with liver and cause toxicity.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ understand reasons of susceptibility of liver to toxic insult;
- ❖ know the functional anatomy of liver;
- ❖ know various pathological conditions caused by toxicants; and
- ❖ learn how various hepatotoxic substances cause damage to liver.

Table 9.1: Overall functions of liver

Metabolism/processing of nutrients (carbohydrate, lipid, amino acids)
Synthesis (gluconeogenesis, lipoproteins, blood proteins, hormones, heme, lymph)
Storage (carbohydrates, vitamins, minerals)
Excretion of xenobiotics in bile
Metabolism of xenobiotics
Immunological functions
Digestion of food

9.2 FUNCTIONS AND SUSCEPTIBILITY OF LIVER TO TOXIC INSULT

Liver is the largest organ and is metabolically more active than any other organ of the body. Liver is also an important organ from toxicological point of view since it is the main organ of metabolism and excretion of xenobiotics (via bile).General functions of liver are given in table 1.

Liver is often a target organ for several toxicants. The reasons for susceptibility of liver to toxic insult are:

i. Liver is the first organ which receives nutrients and xenobiotics absorbed from the intestine via portal vein. Thus, liver is exposed to a high concentration of xenobiotics absorbed from gastrointestinal tract.

ii. Liver has the highest concentration of xenobiotic metabolizing enzymes. In cases wherein toxicants are rendered more toxic or reactive after metabolism, liver is the first organ where these reactive metabolites cause lesions.

iii. Cells of liver (hepatocytes) are in close contact with blood due to fenestrated capillary system in liver. This directly exposes hepatocytes to xenobiotics.

iv. Liver cells have a high number of various membrane transport proteins which can actively and selectively concentrate toxicants.

9.2.1 Functional Anatomy of Liver

Liver receives blood from *i)* intestine via 'hepatic portal vein', which carries absorbed material and, *ii)*'hepatic artery' which supplies oxygenated blood to liver. The blood is drained through central veins which open in hepatic vein. At microscopic level, liver is organized into hexagonal structural units 'lobules' (Fig. 9.1b).The bulk of liver is comprised of 'hepatocytes' which are arranged linearly in each lobule called 'hepatic cords' separated by 'sinusoids' (Fig. 9.1b). Sinusoids are wide capillaries in which the endothelial lining is discontinuous (fenestrated endothelium) allowing free movement of substances from blood to the hepatocytes (Fig. 9.1d). At the corners of lobules lies'portal triad' which contains branches of 'hepatic portal vein', 'hepatic artery'and 'bile duct'.

Sometimes, the microscopic structure of liver is viewed in a different manner– the 'hepatic acinus' is considered the functional unit of liver (Fig.9.1c). This view better explains the way blood flows in the liver at microscopic level. Blood entering via branches of portal vein and hepatic artery mixes and enters into the sinusoids (Fig. 9.1d). Here, blood comes in contact with hepatocytes and exchange of matter takes place between blood and the hepatocytes. The blood from sinusoids slowly moves towards the central vein and finally leaves liver via hepatic vein (Fig. 9.1d). Note that bile in canaliculus flow is in reverse direction because bile is produced by hepatocytes by secreting the components into bile canaliculi. The bile in canaliculi flows towards the bile duct and then pours into the intestinal lumen via a common bile duct (Fig. 9.1 D).

In figure 9.1d, you can make out the main types of cells found in the liver:

1) Hepatocytes

2) Endothelial cells

3) Kupffer cells (macrophages),

4) Hepatic stellate cells (fat-storing cells)

Hepatocytes are the most abundant of all the cell types in the liver (about 60% of total cells) and are responsible for most of the metabolism of nutrients, synthesis and storage of nutrients as given in table 1. Hepatocytes extract or uptake xenobiotics from the sinusoidal blood and metabolize them with the help of xenobiotic metabolizing enzymes. The primary function of Kupffer cells is to engulf and degrade (i.e. phagocytosis) particulate matter (e.g. bacteria) and remove them from the blood.

9.2.2 Liver Zonation

Under the microscope, at structural level, all the hepatocytes of liver look alike. However, there exists a difference in biochemical functions (therefore enzymes, transporters, metabolites, etc.)among hepatocytes arranged along the entire length of hepaticcords in liver acinus (Fig.9.1d). So, on the basis of biochemical functions, and composition of biomolecules in the hepatocytes, acinuscan been divided into three zones, viz. 1, 2 and 3. Let us explain this phenomenon with an example: processes which consume more oxygen or energy (such as protein secretion, gluconeogenesis, beta-oxidation, etc.) are carried out by hepatocytes which are closer to the portal triad i.e. zone 1, whereas less energy or oxygen demanding processes (such as xenobiotic metabolism, glycolysis, ketogenesis, glycogen synthesis, etc.) take place in hepatocytes which are nearer to the central vein or zone 3 (Fig.9.1d). These differences are due to the fact that the oxygen (and nutrients) content in blood closer to the portal triad (blood to which zone 1 hepatocytes are exposed) is higher (60-65 mm Hg); and as the blood moves towards zone 3,metabolic activities in hepatocytes of zone 1 and 2removes oxygen.Blood reaching zone 3 thus has only half the original amount of oxygen.The phenomenon of gradual differences (in the biochemical functions, enzymes, transporters, etc.) in hepatocytes moving from one end to another along the hepatic cord is called 'liver zonation'.

The zonal differences also make one zone more susceptible to toxic insult than another. For example, zone 3 has the highest concentration of CYPs. A toxicant (such as paracetamol) activated by CYPs therefore would cause more damage to zone 3 hepatocytes.Similarly,allyl alcohol causes toxicity in zone 1, because of its higher uptake and activation in this zone.

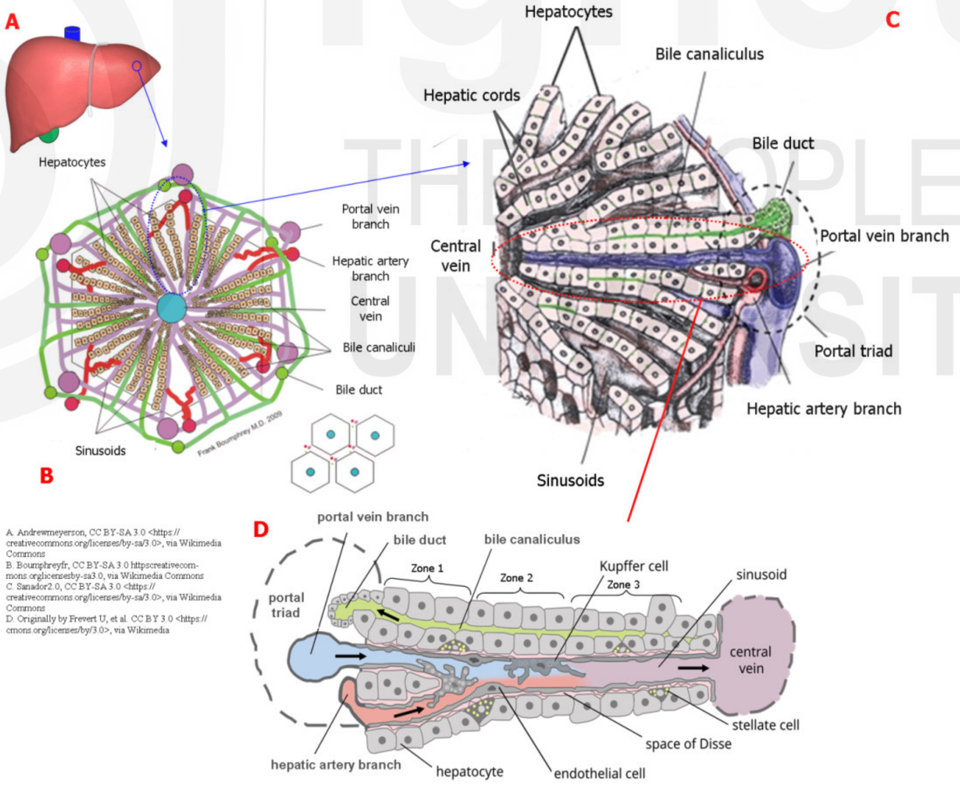

Fig. 9.1: Functional anatomy of liver: (A) external morphology, (B) a 'lobule' showing organization of'hepatocytes' and various 'vessels', (C) 'hepaticacinus' (a portion) showing lineararrangement of hepatocytes– 'hepatic cords', 'sinusoids', 'bile canaliculus' and blood vessels (D) various cells of liver, and direction of flow of blood and bile.

(Source: b) Boumphreyfr, CC BY-SA 3.0 httpscreativecommons.orglicensesby-sa3.0, via Wikimedia Commons d) Wikimedia Commons)

SAQ 1

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

i) Kupffer cells are the most abundant cells in liver (True/False).

ii) Presence of high concentration of metabolizing enzymes makes liver susceptible to toxic insult (True/False).

iii) Zone 1 of hepatic acinus receives highest concentration of oxygen(True/False).

iv) Central vein in the hepatic lobules supplies fresh blood to the liver. (True/False).

b) Fill in the blanks with appropriate words:

i) The function of Kupffer cells is................

ii) Hepatic stellate cells are also known as ....

iii) ....is considered as functional unit of liver.

9.3 TYPES OF LIVER INJURIES

As we discussed above, liver is susceptible to several toxicants. The effects of various toxicants may range from mild reversible cell injury or necrosis to irreversible alteration in microscopic and macroscopic structure of the liver (such as cirrhosis). Extensive damage to the architecture limits liver to perform essential functions leading to liver failure, which may be fatal. We have seen the basic structural plan of liver and major types of cells in the previous section. In this section, we will discuss pathological changes in liver caused by toxicants.

9.3.1 Necrosis

Liver necrosis is death of liver cells, particularly, the hepatocytes. Necrosis may be restricted to a small part of liver and affect only few cells (focal) orinvolve a large area of the liver (massive necrosis). A cell undergoing necrosis swells up and the cellular contents including the enzymes, proteins and metabolites leak out. Appearance of enzymes alanine aminotransferase (ALT) and aspartate amino transferase (AST) in blood has been used to monitor liver damage. Both the enzymes are predominantly found in liver. Necrosis is usually an acute injury and may not be critical due to extraordinary regenerating capacity of liver. Massive necrosis exceeding beyond the capacity to repair leads to serious liver damage or liver failure.

9.3.2 Fatty Liver (Steatosis)

Fatty liver is a condition of excessive deposition of ‘triglycerides’ in the hepatocytes. The depositionof fat droplets displaces nucleus to the periphery of the cell (Fig. 9.2). Fatty liver does not necessarily indicate a state on non-functional hepatocytes and is reversible if the stimulus causing fatty liver is

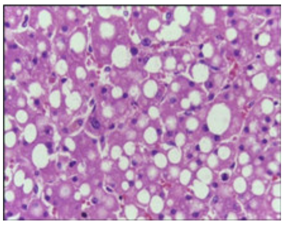

Fig. 9.2: Microscopic view of liver hepatocytes containing fat droplets (white spaces), displacing the nucleus (blue stained) towards periphery.*Image courtesy: Calicut Medical College, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>,via Wikimedia Commons* (Source:Calicut Medical College, CC BY-SA 4.0 httpscreativecommons. orglicensesby-sa4.0, via Wikimedia Commons)

removed. However, accumulation of fat makes liver susceptible to injury by other hepatotoxicants. Fatty liver may progress into a more serious condition–cirrhosis. The reason for accumulation of fat in the liver are: increased supply of fat to the liver from adipose tissue and diet, and decreased metabolism and impaired transport of triacylglycerol from liver to adipose tissue. Liver transports triacylglycerol packed in the form of VLDL (Very Low Density Lipoproteins). VLDLs are vesicles made of various lipids and proteins. Thus, toxicants interfering with the synthesis of VLDL proteins, phospholipids, andmovement of VLDL across cell membrane can cause accumulation of fat in the liver. Ethanol, CCl_4, tamoxifen and valproic acid are few examples of toxicants which promote fat deposition in the liver.

9.3.3 Cholestasis

Refer to figure 1 and follow the direction in which bile flows. Bile is formed when the components of bile (bile salts, conjugated bilirubin, phospholipids, cholesterol, ions, and water) are transported by hepatocytes across the cell membrane into bile canaliculi. The transportation of bile components is facilitated by several membrane transport proteins. The metabolites of xenobiotics are secreted by hepatocytes in the bile by the same membrane transport system. Bile from several bilecanaliculiis eventually poured into the bile duct from where it moves to larger ducts and finally secreted in the intestine via common bile duct. The formation and adequate flow of bile through bile ducts is necessary for normal physiological functions such as digestion and excretion of metabolites of xenobiotics and endogenous substances.

In cholestasis, the flow of bile is reduced, and in extreme situations, the flow stops. It is due to the impairment in secretion of bile components by hepatocytes or obstruction to the flow of bile through ducts. Inflammation and accumulation of dead cell debris in the bile ducts are the reasons for obstruction to bile flow. The accumulating bile components in hepatocytes begin to appear in the blood. The yellowing of skin and eyes due to presence of excess amount of bile pigments in bloodis called 'jaundice'.

9.3.4 Fibrosis and Cirrhosis

Fibrosis (sometimes also called scarring) is replacement of the normal tissue with fibrous tissue, similar to that occur in the wound healing process of skin. Hepatic fibrosis is due to the chronic liver injury caused by toxicants or other factors such as inflammation. During hepatic fibrosis, there is extensive deposition of collagen fibres around the central vein, portal tracts and space of Disse (see Fig. 9.1D). With continuing fibre deposition, an interconnection between scars develops and liver is divided into nodules with hepatocytes. The normal lobular architecture of liver is damaged–a condition known as 'cirrhosis'. The excessive fibre deposition disrupts the characteristic blood flow in liver (as discussed above) which impedes exchange of nutrients and affects normal functioning of liver. Worldwide, viral hepatitis and alcohol abuse are the leading causes of fibrosis and cirrhosis.

SAQ 2

State whether the following statements are true or false:

i) In cholestasis, there is a heavy deposition of fat in the hepatocytes (True/False).

ii) Presence of alanine aminotransferase (ALT) in the blood may indicate ongoing necrosis of hepatocytes (True/False).

iii) Fatty liver may be caused by defect in transport of lipids from liver to adipose tissue (True/False).

iv) Deposition of large amount of cholesterol in hepatocytes causes steatosis (True/False).

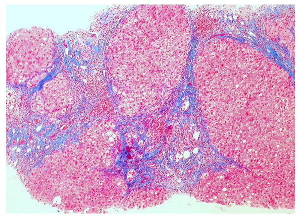

Fig. 9.3: Microscopic view of liver showing interconnected fibrous scars dividing the liver parenchyma.*Image courtesy: Nephron, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons*

(Source: Ed Uthman from Houston, TX, USA, CC BY 2.0 httpscreativecommons.orglicensesby2.0, via Wikimedia Commons)

9.4 HEPATOTOXIC AGENTS

A variety of xenobiotics including drugs, natural poisons such as plant and animal toxins, metals, drugs, food additives, etc. can harm liver. In this section, we will see the mechanism of action of major hepatotoxicants.

9.4.1 Microcystins

Microcystins isa group of toxins produced by ‘cyanobacteria’or ‘blue–green algae’ growing in lakes and reservoirs. Microcystin-LR is the most common and well-studied of all microcystins. Humans and animals are exposed to microcystins by drinking contaminated water. The main target organ of microcystins is liver, although it has also been reported to affect kidneys and intestine. It is taken up by hepatocytes by transporters OATP (organic anion-transporting polypeptides). A higher number of these transporters on hepatocyte cell membrane accounts for selective hepatotoxicity by microcystins (Fig. 9.4).

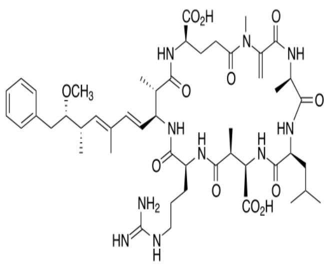

Fig. 9.4: Microcystin-LR.

(Image courtesy: Charlesy, Public domain, via Wikimedia Commons)

Microcystins causes deformity in hepatocytes and their detachment from each other disrupting the normal architecture. Microcystins bind to cysteine residue of the enzymes - protein phosphatases. These enzymes are responsible for removal of phosphate groups from other proteins and enzymes making them active or inactive. Inhibition of phosphatases causes accumulation of phosphorylated proteins such as those involved in cell signalling and cell proliferation.

9.4.2 Amatoxins

It is a group of toxins produced by toxic mushroom *Amanita phalloides*. This toxin is efficiently absorbed from intestine and reaches liver; here,it enters hepatocytes using OATP transporters. It causes necrosis of hepatocytes. Amatoxins are potent inhibitors of RNA polymerase II, which is required for transcription of mRNA. This results in inhibition of protein synthesis and death of the cells (Fig. 9.5).

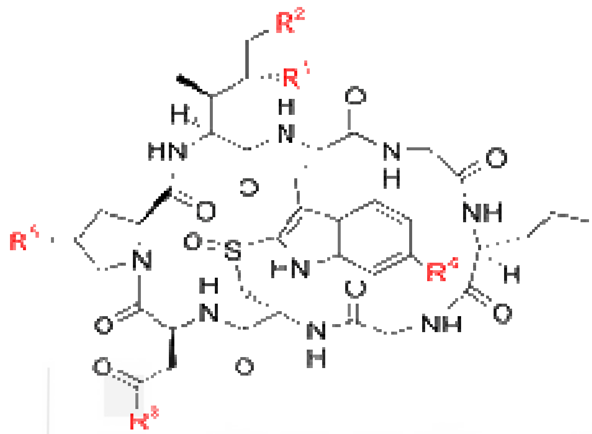

Fig. 9.5: Backbone structure of Amatoxins.
(Image courtesy:Edgar181, Public domain, via Wikimedia Commons)

9.4.3 Carbon Tetrachloride

It is a potent and well-studied hepatotoxicant. CCl_4 is metabolized in liver by CYP450 to trichloromethyl radical ($^{\bullet}CCl_3$), which after reaction with oxygen, gets converted to trichloromethylperoxyl radical ($^{\bullet}OOCCl_3$). Both the free radicals are highly reactive and can react with proteins in hepatocytes and initiate lipid peroxidation (Fig.9.6). The latter is the process by which free radicals can fragment membrane lipids and damage cell membrane. Damage to cell membrane causes leakage of cellular contents and finally necrosis. Continued exposure to CCl_4causes extensive necrosis and replacement of hepatocytes with fibrous mass–fibrosis.

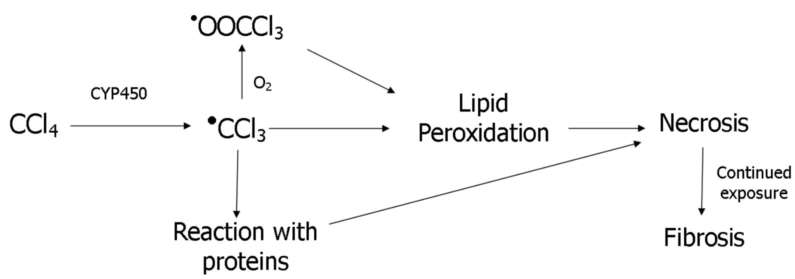

Fig. 9.6: Mechanism of hepatotoxicity by carbon tetrachloride.
(Source: CCL4 toxicity Rajesh Chaudhary)

9.4.4 Paracetamol

It is a safe drug and available as over-the-counter drug for relieving pain and fever. However, overdosing of paracetamol can cause serious liver injury which may be fatal if not intervened. Paracetamol poisoning causes death of hepatocytes. Refer to fig. 9.7, under normal or therapeutic dose, most of paracetamol in metabolized in the liver via glucuronidation and sulfonation reactions and the harmless metabolites are excreted in bile and urine. However, in the event of overdosing, the two enzymes (see figure) getsaturated and a significant amount of paracetamol is metabolized by CYP450 to produce a toxic metabolite called N-acetyl*p*-

benzoquinone imine (NAPQI). This metabolite is detoxified by glutathione conjugation, which also gets depleted on increased production of NAPQI. Thus, overdosing causes accumulation of NAPQI in hepatocytes that reacts with proteins and initiates toxicity.

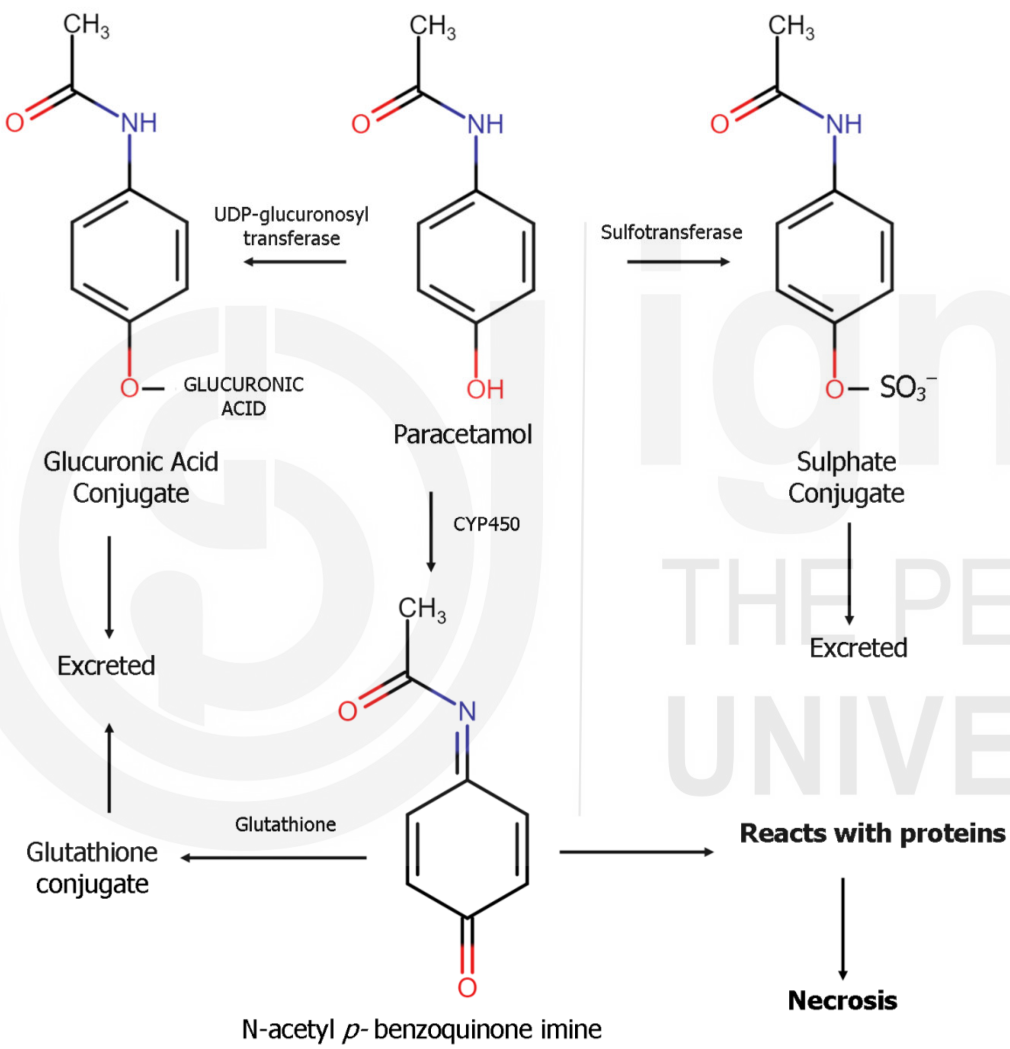

Fig. 9.7: Mechanism of hepatotoxicity by paracetamol.

(Source: PCM metabolism Rajesh Chaudhary)

9.4.5 Ethanol

Chronic ethanol abuse is a leading cause of liver damage. Ethanol is metabolized by three pathways using enzymes (CYP450, alcohol dehydrogenase and catalase) and in each of thesecases the product is acetaldehyde, which is metabolized to acetate by aldehyde dehydrogenase. Acetate is converted to water molecule and carbondioxide via Krebs cycle.

As we have seen above, excessive or chronic alcoholism causes fatty liver or steatosis. Although the mechanism leading to fat deposition in liver and subsequent events leading to cirrhosis is complex, it may be summarized as follows: *i)* ethanol increases cellular supply of acetate and NADPH which increase triacylglycerol synthesis, *ii)*the acetaldehyde formed from ethanol inhibits proteins involved in the formation and secretion of VLDL. This prevents transport of triacylglycerol from liver to adipose tissues. Both the above steps cause accumulation of triacylglycerol in the liver, which is augmented by the habit of consuming a diet rich in fats. Steatos is makes liver susceptible to several pathological injuries andtoxicants, but is not considered enough to cause liver failure.

Continued alcoholism brings in involvement of immune system and necrosis of hepatocytes which is as follows: metabolism of ethanol by CYP450 (as discussed above) causes generation of free radicals which causes necrosis of hepatocytes. Further, acetaldehyde can directly stimulate fibre deposition by stellate cells and also activate Kupffer cells. In summary, necrosis of hepatocytes and stimulation of stellate cells causes fibrosis; that in extreme cases, leads to cirrhosis and liver failure.

9.4.6 Allyl Alcohol

This industrial chemical is preferentially taken up by the hepatocytes of zone 1. It is metabolized by alcohol dehydrogenase to a reactive metabolite 'acrolein', which reacts with the proteins in hepatocytes and can initiate lipid peroxidation (Fig. 9.8). These events lead to necrosis of hepatocytes. A reduced level of glutathione in the hepatocytes aggravates liver injury because acrolein is detoxified by conjugation with glutathione.

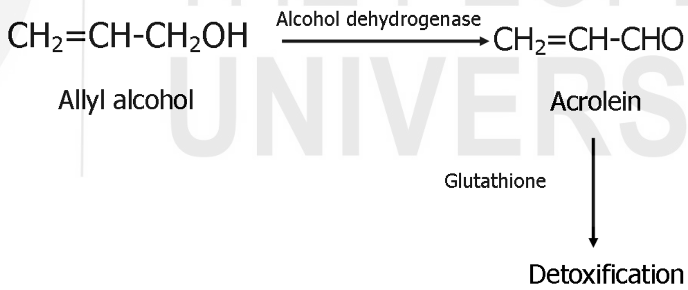

Fig.9.8: Activation of allyl alcohol to acrolein by alcohol dehydrogenase in liver. (Source: acrolein Rajesh Chaudhary)

SAQ 3

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

i) Exposure to carbon tetrachloride causes lipid peroxidation in hepatocytes (True/False).

ii) Under normal therapeutic dose, significant amount of paracetamol is converted to NAPQI which is responsible for necrosis of hepatocytes(True/False).

iii) Liver is susceptible to amatoxins because they can be selectively accumulated in hepatocytes (True/False).

b) Fill in the blanks with appropriate words:

i) Toxic metabolite of paracetamol is produced by the enzyme.................

ii) Toxic metabolite of allyl alcohol is produced by the enzyme................

iii) Amatoxins are inhibitors of ....

9.5 SUMMARY

- Liver is the largest and most active organ of the body. It performs several functions which are crucial for the survival. There are several toxicants (hepatotoxicants) which specifically damage structure of liver and can cause liver failure.

- Liver is the main organ for xenobiotic metabolism and receives a high concentration of toxicants absorbed from the site of exposure (particularly gastrointestinal tract). Liver contains a high concentration of various xenobiotic metabolizing enzymes and transporters. These factors make liver susceptible to toxic insult.

- Liver has an ordered arrangement of cells and a unique manner of blood flow which is essential for the function of liver. The most abundant cells in liver are the hepatocytes which are arranged linearly around wide capillaries called sinusoids. Hepatocytes perform most of the functions of liver and are main target of toxicants.

- Chronic exposure or exposure to high concentrations of toxicants can cause necrosis or death of hepatocytes, which is repairableup to a certain limit. Massive death of hepatocytes and their replacement with collagen fibres is called fibrosis. Extensive fibrosis may progress to a more serious condition called cirrhosis, in which the liver is divided by fibrous septa into nodules enclosing hepatocytes. This damages the normal functional architecture of the liver and may lead to liver failure.

- Toxicants such as paracetamol, allyl alcohol, carbon tetrachloride can cause acute necrosis, whereas chronic consumption of ethanol can cause steatosiswhichmay progress to fibrosis and cirrhosis.

9.6 TERMINAL QUESTIONS

1. Provide mechanism of action of hepatotoxicity by paracetamol.
2. Explain why liver is susceptible to toxic insult.
3. Briefly describe structure of a hepatic lobule.

9.7 ANSWERS

Self-Assessment Questions

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

   b) i) Phagocytosis

      ii) Fat-storing cells

      iii) Hepatic acinus

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

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

   b) i) CYP450

      ii) Alcohol dehydrogenase

      iii) RNA polymerase II

Terminal Questions

1. In cases of overdosing,paracetamol is metabolized by CYP450 to produce toxic metabolite N-acetyl*p*-benzoquinone imine (NAPQI). This reactive metabolite is detoxified by glutathione conjugation.However,increased production of NAPQI due to overdosing of the drug depletes glutathione reserve,causing accumulation of NAPQI in hepatocytes which can react with proteins and cause necrosis.

2. The reasons of susceptibility of liver to toxicants are as follows:

   i) Liver receives a high concentration of toxicants absorbed from gastrointestinal tract.

   ii) Liver has the highest concentration of xenobiotic metabolizing enzymesand in the event of metabolic activation; the activated xenobiotics immediately react and damage the cellular components of hepatocytes (cell membrane lipids, proteins and DNA).

3. Hepatic lobules are hexagonal structures, the boundary of which is limited by a thin bundle of connective tissue. Lobule is the structural unit of liver. Withina lobule,hepatocytes are arranged in a linear manner called hepatic cords. Along the length of cords liesa wide blood-filled cavity known as hepatic sinusoid. On the corners of lobules,there are hepatic triads–a collection of vessels such as branches of hepatic artery, hepatic portal vein, and bile duct. In thecentre of lobule,there is a central vein which collects blood flowing from vessels of hepatic triadthrough sinusoids.

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Unit 10

NEPHROTOXICITY

Structure

10.1 Introduction

Expected learning Outcomes

10.2 Functions and structure of Kidneys

Main Functions and Susceptibility of Kidneys to Toxic Insult

Functional Anatomy of Kidneys

10.3 Toxicant induced injury to kidneys

Overview

Acute Kidney Injury (AKI)

Regenerationof Nephrons

10.4 Nephrotoxic agents

Chloroform

Paracetamol

Amphotericin B

Cyclosporine

Cisplatin

Mercury

Cadmium

Ethylene Glycol

10.5 Summary

10.6 Terminal Questions

10.7 Answers

10.8 Further Readings

10.1 INTRODUCTION

The main function of kidneys is to remove wastes or substances to be excreted from the blood. The ability of kidneys to concentrate toxicants in the renal tubules or nephrons, and a high blood flow makes the cells of kidneys susceptible to toxic insult. The target site of most toxicants in kidneys is renal tubules and blood vessels. Toxicants may also cause vasoconstriction and reduce blood flow in the kidney. This results in hypoxic injury and reduction in GFR (Glomerular Filtration Rate). Many toxicants that damage renal tubules, do so in a site-specific manner. In this Unit, we will study the functional anatomy of kidneys, toxic injury and mechanism of toxicity of a few main toxicants that damage kidneys.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ Understand reasons of susceptibility of kidneys to toxic insult;
- ❖ know the functional anatomy of kidneys;
- ❖ know about toxicant induced damage to kidneys; and
- ❖ learn how various hepatotoxic substances cause damage to kidneys.

10.2 FUNCTIONS AND STRUCTURE OF KIDNEYS

Kidneys perform several important functions for the body. A variety of toxicants can harm kidneys and thereby change the normal physiology of the body. Inthis section, we will discuss the main functions and reasons for the susceptibility of kidneys to various toxicants.

10.2.1 Main functions and Susceptibility of kidneys to Toxic Insult

Kidneys remove waste/toxic metabolites produced after metabolism of endogenous substances and xenobiotics and excrete them from the body in urine. In addition, kidneys also perform several other crucial functions such as acid-base and osmotic balance, regulation of extracellular fluid volume, synthesis and secretion of hormones(such as renin and erythropoietin), and conversion of vitamin D into its active form. Damage to kidneys makes it impossible for the body to remove waste substances from blood. Presence of high concentration of some of these excretory substances in the blood are used to diagnose damage to the kidneys.

Kidneys are the major target organ for toxicants–a wide range of toxicants (including, environmental pollutants, drugs, toxins, metals, etc.) can damage kidneys and thus, prevent them from performing vital functions. The following factors make kidneys susceptible to toxic insult:

i. Kidneys receive 25% of the cardiac output, thus, various cells of kidneys are continuously exposed to a high amount of toxicants carried by blood.

ii. During the formation of urine, kidneys concentrate the contents of tubular fluid to prevent water loss in urine. Thus, the concentration of a xenobiotic which is below toxic level in blood may increase to toxic levels in the renal tubules.

iii. The extensive membrane transport system, ability to accumulate certain toxicants, and ability to metabolize xenobiotics, together increases susceptibility of kidneys to toxicants.

10.2.2 Functional Anatomy of Kidneys

The interior of kidneys is divided into three parts: the outer ‘cortex’, middle ‘medulla’, and the innermost ‘papilla’ (Figure 1A and B). *Cortex* constitutes the major part of the kidneys; this area contains ‘renal glomeruli’, ‘proximal tubules’ and ‘distal tubules’ of nephrons, and receives about 90% of the blood that flows to the kidneys. *Medulla*,which receives about 6% of the renal blood flow, is organized into several inverted pyramid-shaped structures called‘renal pyramids’. The part of nephrons lying in the medulla includes ‘loops of Henle’, and ‘collecting ducts’. The apical part of the renal pyramids is called ‘renal papilla’ (Fig. 10.1 a and b). The renal *papilla*r eceives about 1-2 % of the renal blood flow and this area contains ‘papillary duct’ of nephron. The urine formed in the nephrons is delivered to ‘renal calyces’ via papillary ducts, from where urine flows to ‘ureter’ and leaves the kidney. Note that medulla and papilla receive a lesser amount of blood and thus, are susceptible to ischemia and hypoxic injury produced by toxicants (we will discuss below).

After getting an idea of the gross anatomy of kidneys, let us have a look at the network of blood vessels in the kidneys and the pattern of blood flow.

Refer to figure 10.a and 10.b, where blood to be filtered enters kidneys via ‘renal artery’. The renal artery branches into narrower vessels and finally enters a cup shaped structure–‘Bowman's capsule’ as ‘afferent arteriole’. The afferent arteriole forms a network of capillaries in the Bowman's capsule called ‘glomerulus’. At the other end, the capillaries of glomerulus combine and leave Bowman's capsule as ‘efferent arteriole’. The efferent arteriole divides into a network of capillaries (called ‘peritubular capillaries’) around a nephron. The peritubular capillaries then unite and join a branch of renal vein. The latter takes away filtered blood out of the kidney.

Now that we have seen the gross anatomy of kidneys and blood flow, let us see the structure of a ‘nephron’ which is also called a ‘functional unit’ of kidneys.

Refer to the right most diagram of fig. 10.b, you will find that each nephron has the following parts: Bowman’s capsule, proximal tubule,loop of Henle, distal tubule, and collecting tubule. The ‘renal tubule’ is a collective term used to denote proximal tubule, loop of Henle, distal tubule and collecting tubule.

Bowman's capsule is a cup shaped structure in which afferent arteriole forms a network of capillaries (glomerulus). The glomerulus is intimately covered by flat cells called ‘podocytes’ (Fig. 10.c). It is the glomerulus where blood is filtered and the ‘filtrate’ (called glomerular filtrate) flows into the next part of kidney –proximal tubule.

Proximal tubule: This part of nephron reabsorbs about 70-80% of the solute (nutrients, minerals, etc.) filtered in the glomerular filtrate. The epithelial cells lining the proximal tubule actively reabsorb and secrete solutes in the tubular lumen. The two processes are enabled by different types of transporter proteins in their cell membrane. To drive transport of solutes across their cell membrane, energy and oxygen requirement of epithelial cellsof proximal tubule is high. The proximal tubule is therefore susceptible to toxicants interfering with the production of energy, and those inhibiting membrane transporters and enzymes involved in transportation of solutes across cell membrane.

Loop of Henle: This part of nephron is concerned with the concentration of urine by absorbing water and salts (particularly sodium and potassium) which require a high amount of energy. Less supply of blood to medulla (see above), makes this part of nephron susceptible of hypoxic injury caused by toxicants.

Distal tubule and collecting duct: This part of nephron reabsorbs remaining sodium and water. The hormone ADH (Anti Diuretic Hormone) helps reabsorption of water by increasing permeability of collecting ducts for water. Therefore, toxicants that hinder synthesis, secretion of ADH by pituitaries, and action of ADH on collecting ducts can increase loss of water in urine.

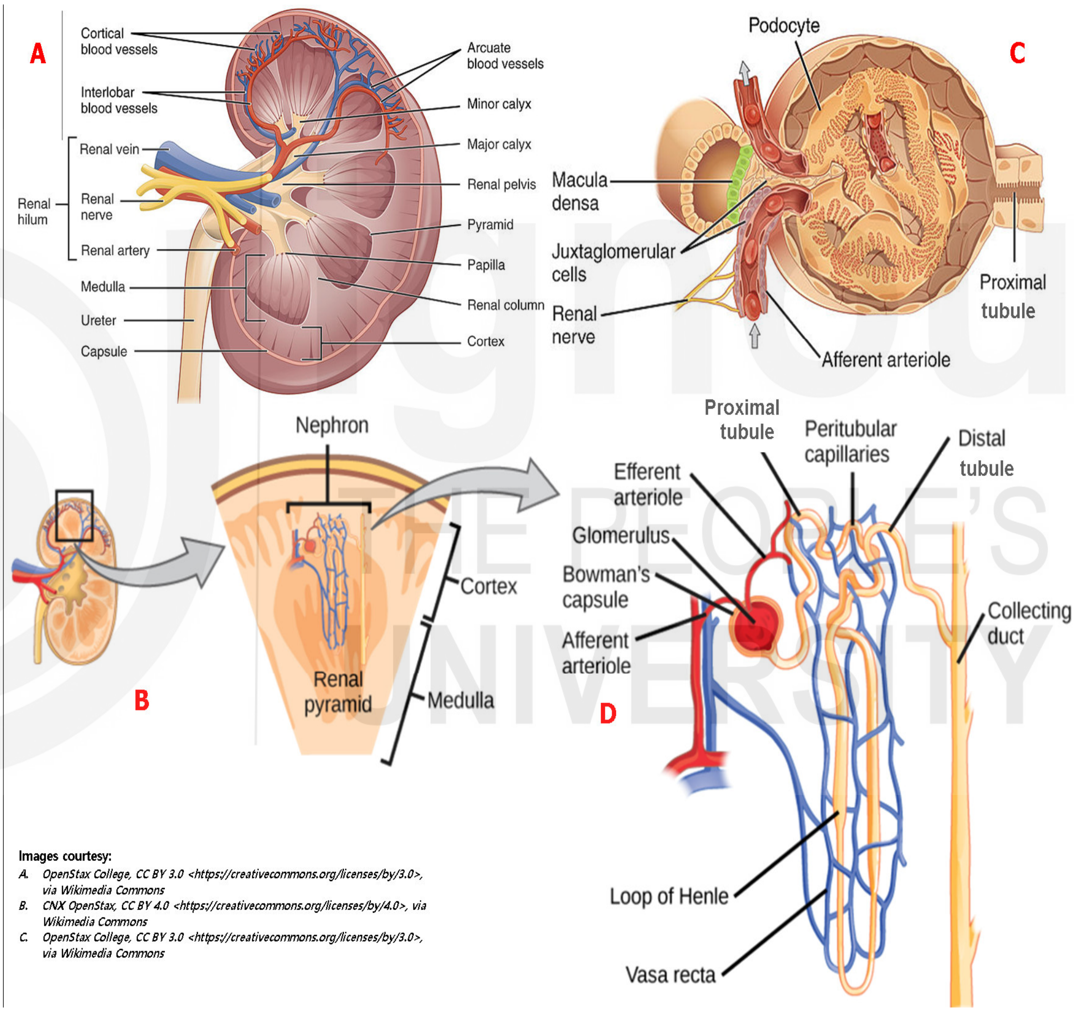

Fig. 10.1: Functional anatomy of kidney; (A) gross anatomy showing various parts of kidney; (B) showing position of nephrons in kidney; (C) a glomerulus showing glomerular capillaries covered intimately by podocytes and; (D) various parts of a nephron and blood supply to the nephron.

(Source: a) OpenStax College, CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons b) CNX OpenStax, CC BY 4.0 httpscreativecommons.orglicensesby4.0, via Wikimedia Commons c) OpenStax College, CC BY 3.0 httpscreativecommons.orglicensesby3.0, via Wikimedia Commons)

SAQ 1

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

i) Podocytes are found wrapped around glomerular capillaries (True/False).

ii) The medulla of kidneys receives highest amount of blood (True/False).

iii) Kidneys are susceptible to toxic insult because they receive high blood flow and the concentration of toxicants can increase in the renal tubules during the formation of urine. (True/False).

b) Fill in the blanks with appropriate words:

i) The part of nephron where blood gets filtered is.................

ii) The parts of the kidneys .... and ....are susceptible to hypoxic injury.

10.3 TOXICANT INDUCED INJURY TO KIDNEYS

In this section, we will briefly discuss the changes in the architecture and function of kidneys in response to toxic insult.

10.3.1 Overview

As we have seen above, kidneys have two main systems: i) a unique arrangement of blood vessels and blood flow and, ii) nephron i.e. the system which removes excretory waste products from blood and preserves minerals and other nutrients. The two systems work in coordination with each other and thus, a fault in one, may affect the performance of the other.

The overall effect of kidney damage is the retention of waste products/metabolites in the blood. The elevated levels of these waste products in the blood can affect normal physiology directly or indirectly, and lead to a life-threatening situation.

There are several ways by which toxicants affect kidneys. For simplicity, we can group toxic effects into: i) those occurring in blood vasculature of kidneys, and ii) those that directly damage nephrons.

Toxicants affecting blood flow and vasculature, such asprolonged use of NSAIDs, (Non-Steroidal Anti-Inflammatory Drugs) can cause vasoconstriction(reduced blood flow due to narrowing of blood vessels) in the kidneys and create a hypoxic condition particularly in medulla and papilla(since these areas receive less blood, see above). Secondary to this effect is the death of epithelial cells lining the loop of Henle, a reduced GFR (Glomerular Filtration Rate) and retention of waste metabolites in the blood.

Many toxicants that *directly damage nephrons* do so in a site-specific manner, i.e. they affect or act on a particular part of the nephron. The reason for this site-specific action is differences in certain biochemical properties of epithelial cells lining different parts of neurons such as: presence of specific transporters, xenobiotic metabolizing enzymes and other enzymes, ability of toxicants to get accumulated in the cells, energy demand of the cells, and flow of blood. For example, lead is taken up by epithelial cells lining the proximal tubule and causes damage to this part of nephron, whereas puromycin causes death of podocytes (see Fig. 10.1c) leading to loss of glomerular function.

Proximal tubule is the most common site where toxicants act. The susceptibility of this segment of nephron is due to the presence of varieties of transporters; as well as high activity of CYP450 and other metabolizing enzymes which can activate toxicants. Further, proximal tubules are more sensitive to toxicants which reduce production of energy in the cells or produce a hypoxic condition.

10.3.2 Acute Kidney Injury (AKI)

It refers to a sudden reduction in the functions of the kidneys (reduction in GFR), and is also denoted as Acute Renal Failure (ARF). Acute Renal failure is usually irreversible. Toxicant induced Acute Kidney Injury (AKI) may be due to: a decrease in renal flow (caused by drugs such as amphotericin B), injury to glomeruli, or due to acute tubular necrosis (Fig.10.2) leading to blockage of tubular lumen (caused by CCl_4, ethylene glycol, lead, etc.).In tubular necrosis, dead cells detach from epithelial layer and block the tubular lumen causing backflow of tubular fluid (or filtrate). This increases the intra-tubular pressure and causes a resultant decrease in GFR.

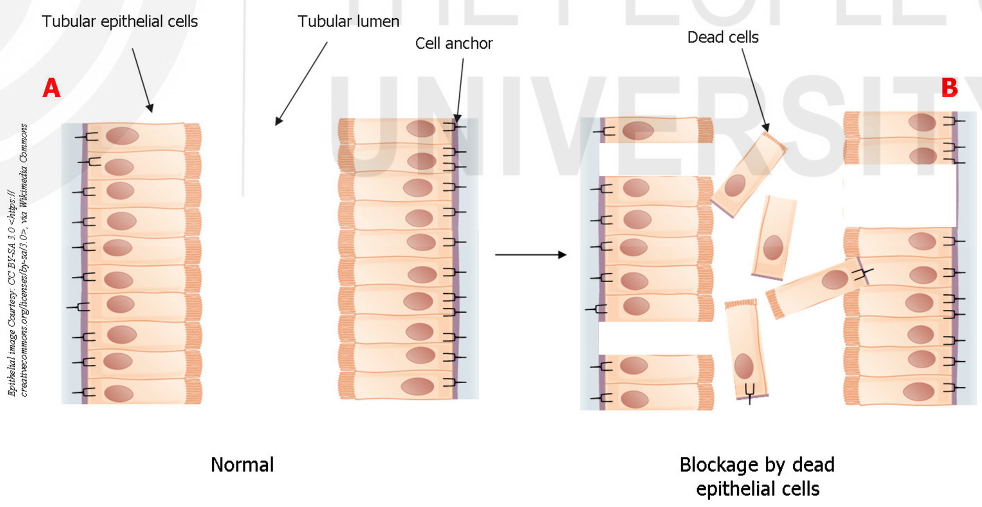

Fig. 10.2 : Cell death in the tubular lumen of nephron; (A) normal tubular lumen and; (B) dead cell debris blocking the tubular lumen.

10.3.3 Regeneration of Nephrons

Kidneys have ability to regenerate–repair the injured cells and replace the cells lost in the tubules due to toxic insult or other causes. The cells which are not severely affected undergo repair, whereas mature unaffected cells can undergo hypertrophy and proliferation. Proliferation of mature unaffected cells and ‘progenitor cells’ causes replacement of cells lost due to toxicant induced cell death. The entire process of repair and proliferation helps restoration of the normal function of kidneys. The kidneys, however, cannot increase their number of nephrons or replace the lost ones. The regenerative power of kidney cells decreases with age, and therefore, elders are more susceptible to kidney failure.

SAQ 2

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

i) Kidneys have enormous power to increase the number of nephrons(True/False).

ii) Some toxicants can cause vasoconstriction in the kidneys which results in degeneration of nephrons(True/False).

b) Fill in the blanks with appropriate words:

i) The part of nephron which is most sensitive to toxic insult is.................

ii) Cells which are supposed to replace lost epithelial cells in renal tubules are........... and....................

10.4 NEPHROTOXIC AGENTS

Nephrotoxic agents include drugs, fungal toxins, heavy metals and halogenated hydrocarbons, etc. Nephrotoxicity limits the clinical use of several drugs such as amphotericin B, cisplatin, cyclosporine, etc. In this section, let us see a few main nephrotoxic agents and their mechanism of toxicity.

10.4.1 Chloroform

Chloroform primarily affects proximal tubules of nephrons, resulting in appearance of protein and glucose in urine; and an increase in BUN (Blood Urea Nitrogen). Chloroform is metabolized to the reactive metabolite 'phosgene', which is detoxified by conjugation with glutathione. Excessive exposure to chloroform depletes cellular glutathione reserve. Phosgene reacts with cellular components such as proteins and lipids causing necrosis of the cells (Fig. 10.3).

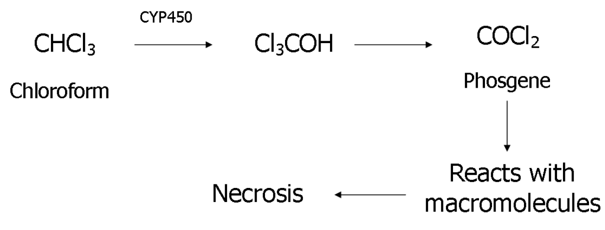

Fig. 10.3: Mechanism of nephrotoxicity by chloroform.

10.4.2 Paracetamol

We have seen in Unit 9 that overdoing of paracetamol causes hepatotoxicity. Paracetamol overdosing can also cause nephrotoxicity simultaneously. As in the case of liver, CYP450 activates paracetamol to N-acetyl *p*-amino-benzoquinoneimine (NAPQI) which reacts with proteins in the epithelial cells of proximal tubulesleading to necrosis of the cells. The toxicity is characterized by increase in BUN and creatinine, decreased GFR, and increase in salt excretion.

10.4.3 Amphotericin B

It is an antifungal drug, with serious side effects of damage to the kidneys. The latter property limits its use as a therapeutic agent. It shows toxic effects by acting on blood vessels of kidneys (reduces renal blood flow and GFR due to vasoconstriction) and nephrons (damages glomeruli and proximal tubules).The property of amphotericin B to bind with cholesterol of cell membrane partly explains its toxic effects (Fig. 10.4).

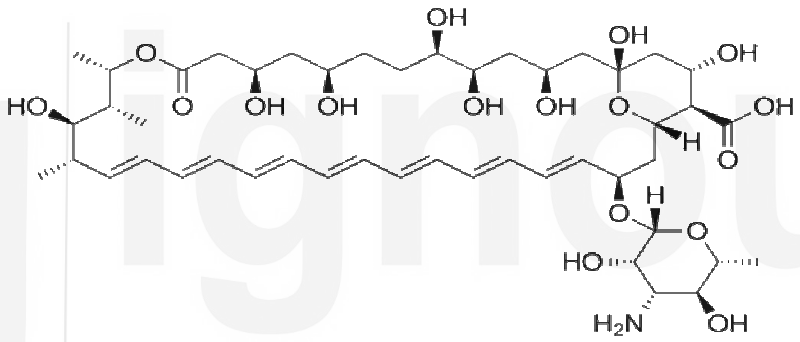

Fig.10.4: Amphotericin B.

(Source: The chemistds, CC0, via Wikimedia Commons)

10.4.4 Cyclosporine

It is an immunosuppressant used after organ transplant to prevent graft rejection and to treat autoimmune diseases. Nephrotoxicity is the main side effect of this drug–it causes vasoconstriction in kidneys resulting in decrease in renal blood flow and GFR. Prolonged use of cyclosporine causesnecrosis andtubular atrophy due to oxidative stress (Fig. 10.5).

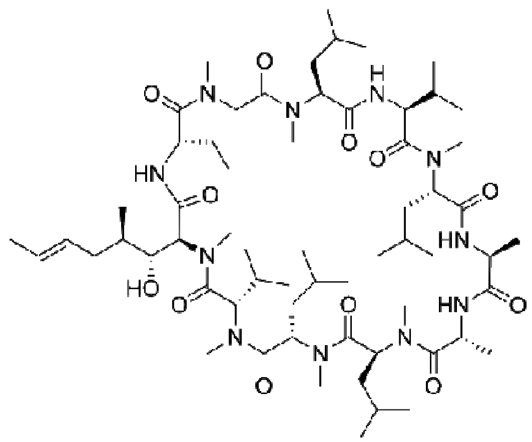

Fig.10.5: Cyclosporine.

(Source: Yikrazuul, Public domain, via Wikimedia Commons)

10.4.5 Cisplatin

Nephrotoxicity caused by this antineoplastic drug is characterized by increased serum creatinine levels and BUN; and appearance of glucose, protein in urine. The part of nephron affected by cisplatin includes proximal tubule and collecting ducts. Cisplatin is taken up by epithelial cells of nephron by ‘organic anion transporter’; whereinit reacts with genomic and mitochondrial DNA and forms intra-strand cross-linkings. This leads to cell death by necrosis or apoptosis.

10.4.6 Mercury

It is an environmental toxicant and humans are exposed to various forms of mercury, viz. Hg^{2+} (inorganic mercury), CH_3-Hg (organic mercury) and Hg^0 (elemental mercury). Elemental mercury is converted to inorganic mercury in the body, and the latter form conjugates with the –SH (thiol) containing molecules such as proteins (Prot–Hg), cysteine (Cys–Hg) and glutathione (GS–Hg). Kidneys are the main target organ of mercury toxicity. The epithelial cells of proximal tubules take up various conjugates of inorganic mercury with the help of amino acid transporters, Organic Anion Transporters (OATs) and other transporter proteins. Since inorganic mercury has high affinity for thiol groups, the former reacts with the thiols of proteins present inside proximal tubule and affect their normal function. Acute nephrotoxicity by mercury is characterized by increase in amino acid, glucose, and salt and protein excretion in urine.

10.4.7 Cadmium

Humans are exposed to cadmium (Cd) through food. It accumulates and shows toxic effect in proximal tubules. The toxicity is characterized by proximal tubular injury and appearance of cellular enzymes, glucose, amino acids and calcium in urine. Cadmium is converted to Cd–metallothioneincomplex in liver which reaches kidneys via blood and is taken up by epithelial cells of proximal tubules via endocytosis and through metal ion transporters. Inside the cell, the unbound or free Cd (Cd not bound to metallothionein) can initiate oxidative stress, cell signalling process and disrupts cell-cell adhesion.

10.4.8 Ethylene Glycol

It is an industrial chemical used for varieties of purposes such as automobile antifreeze and in de-icing formulations. Ingestion is the most common route of exposure to ethylene glycol. It is metabolized in liver to oxalic acid via the pathway shown in figure 10.6. The calcium-oxalate crystals reaching kidneys get deposited in kidney tubules causing necrosis of tubular cells.

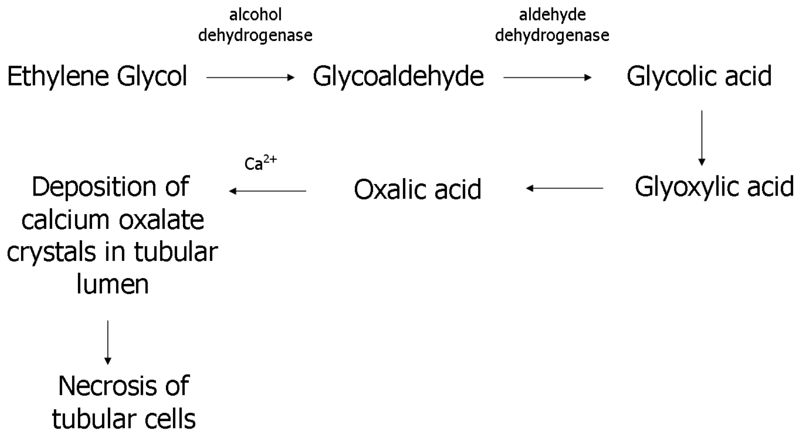

Fig.10.6: Metabolism of ethylene glycol to oxalic acid and resultant injury to kidneys.

SAQ 3

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

i) Cd- metallothionein complex reacts with cellular components in kidneys to show toxicity(True/False).

ii) Cisplatin can crosslink DNA and thus, cause necrosis of tubular cells (True/False).

b) Fill in the blanks with appropriate words:

i) The metabolite of paracetamol named.................. is responsible for nephrotoxicity

ii) The metabolite of chloroform responsible for nephrotoxicity is..................

10.5 SUMMARY

Let us summarize what we have learnt so far:

- Kidneys perform several functions, of which the most important function is to remove metabolic waste products from the blood. Kidneys are the primary organ for excretion. Kidneys are susceptible to toxic insult because they receive large volumes of blood and thus, considerable amounts of toxicants and their metabolites too. Further, during the formation of urine, kidneys concentrate the contents of tubular fluid, and thus, the concentration of a xenobiotic which is below toxic levels in blood, may increase to toxic levels in the renal tubules.
- The functional parts of kidneys are composed of ‘nephrons’– involved in filtration of blood and formation of urine; and‘blood vessels’ that supply blood to nephrons and take away filtered blood to the rest of the body. Nephron, the functional unit of kidneys is divided into: glomerulus,

proximal tubule, loop of Henle, distal tubule, and collecting tubule. Blood is filtered in glomerulus, and during its passage through the tubules, nutrients, ions and water are reabsorbed and most of the waste substances are passed on as urine. Proximal tubule is the most active part of nephron and an important site for the reabsorption of nutrients and water. For optimal performance of kidneys, blood must flow through the vessels in the kidneys at a particular rate. An obstruction or a very high blood flow impairs the function of nephrons.

- Kidneys are the primary target organ of toxicity for various toxicants (including environmental pollutants, drugs, toxins, etc.). Due to high energy demand and presence of various membrane transporters and metabolizing enzymes, proximal tubules are the target site for most of the nephrotoxicants. Toxicants can damage cells of the kidney tubules by reducing blood flow (and resulting hypoxia), and by directly injuring and killing cells. Injury to kidney is reversible if dead cells are replaced by new cells. The new cells are produced by certain progenitor cells and mature cells of kidney tubules.
- Acute Renal Failure (ARF) is a condition of sudden decline in the function of kidneys. Toxicants induced ARF is due to acute necrosis of tubular cells. The dead cells in renal tubules prevent flow of tubular fluid (or filtrate) causing decrease in GFR.
- Majornephrotoxicants include NSAIDs, antimicrobial and antineoplastic drugs, heavy metals such as mercury, cadmium and lead, industrial chemicals such as carbon tetrachloride, chloroform and ethyleneglycol. Many of these toxicants are able to target kidneys because of their selective uptake by kidney tubules, or ability of kidneys to produce their toxic metabolites.

10.6 TERMINAL QUESTIONS

1. Provide mechanism of nephrotoxicity by ethylene glycol.
2. What is Acute Renal Failure? Discuss its causes and consequences.
3. Proximal tubule is the target site for several nephrotoxicants. Justify.

10.7 ANSWERS

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

   b) i) Glomerulus ii) Loop of Henle, Medulla

2. a) i) False ii) True

   b) i) Proximal tubule

   ii) Mature unaffected cells and Progenitor cells

3. a) i) False ii) True

   b) i) NAPQI ii) Phosgene

Terminal Questions

1. Humans are exposed to ethylene glycol via ingestion. Most of the ingested ethylene glycol is metabolized in liver to glycoaldehyde by alcohol dehydrogenase. Glycoaldehyde is metabolized to glycolic acid by aldehyde dehydrogenase. The final product of ethylene glycol metabolism in liver is oxalic acid. The latter metabolite reaches kidney tubules where it precipitates as calcium-oxalate crystals and causes necrosis in tubular cells and blockage in flow of tubular fluid.

2. Acute Renal Failure (ARF) refers to the sudden reduction in the functions of kidneys. A sudden reduction in GFR is indicative of ARF. There can be several causes for AKR, however, toxicant induced AKR is due to decrease in renal flow, injury to glomeruli, and acute tubular necrosis resulting in blockage of tubular lumen. In tubular necrosis, epithelial cells dying due to toxic insult are detached and block the tubular lumen. This prevents movement of tubular fluid which increases the intra-tubular pressure and leads to a decrease in GFR.

3. The susceptibility of proximal tubule to a toxicant is due to: presence of varieties of membrane transporters and enzymes, high activity of CYP450 and other metabolizing enzymes which can activate toxicants. Further due to high energy demand, proximal tubules are more sensitive to those toxicants which reduce production of energy in the cells or produce a hypoxic condition in the kidneys.

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Unit 11

CARDIOVASCULAR AND NEUROTOXICITY

Structure

11.1 Introduction

Expected Learning Outcomes

11.2 Cardiovascular toxicity

Structure of Heart and Blood Vessels

Toxic Responses of Cardiovascular System

Toxic Agents Acting on Cardiovascular System

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Susceptibility of Nervous System to Toxic insult

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11.1 INTRODUCTION

Cardiovascular system and nervous system are crucial for the survival. Due to their unique biochemical features such as high energy demand, and dependence on aerobic respiration for energy, the cells of these two systems are sensitive to toxic induced injury. Several toxicants including drugs, industrial and environmental toxicants, natural toxins can interact with the macromolecules and affect the biochemical processes unique to cardiovascular and nervous systems. Both the organs have little or no ability to replace cells dying due to toxic insult, which causes pathological changes in the structure, and consequently, the function. In this unit, we will discuss functional anatomy of cardiovascular system, and features of nervous system which are important form toxicological point of view. We will also see a few cardiovascular and neurotoxicants and their mechanism of toxicity.

Expected Learning Outcomes

After studying this unit, you should be able to:

- basic features of cardiovascular and neurotoxicity;
- susceptibility of the two systems to toxic insult; and
- main cardiovascular and neurotoxicity agents and their mechanism of toxicity.

11.2 CARDIOVASCULAR TOXICITY

Cardiovascular toxicity is concerned with the adverse effects of toxicants on 'heart' and 'blood vessels' or 'vascular system'. The rhythmic contraction of heart pumps blood in the blood vessels which is carried by the latter to the distant organs. These two subparts of cardiovascular system (i.e. heart and vascular system), work together and ensure adequate perfusion of various organs of the body i.e. delivery of nutrients and oxygen to the organs and removal of waste substances. The ultimate response of cardiovascular system to toxic insult is failure to supply adequate amount of blood to various organs to meet metabolic demands.

Let us see the basic structures of heart and blood vessels.

11.2.1 Structure of Heart and Blood Vessels

Heart*:* rhythmical contraction of the heart is crucial for its functioning. This is achieved by a coordinated action of the two main functional divisions of heart i.e. i) the system of self-generation and spread of electrical impulse (SA node, internodal pathways, AV node, 'bundle of His' and 'Purkinje fibres'), and ii) the system of muscles (cardiac muscles or cardiomyocytes) which contract in response to the electrical impulse. The self-excitatory cells of SA node (sinoatrial node) generate and spread impulse to atria causing atrial muscles to contract. The impulse then reaches AV node (atrioventricular) from where its spreads throughout the ventricles via Purkinje fibres causing ventricular muscles to contract (Fig. 11.1A). There is a slight delay in propagation of impulse from atrium to ventricles. This allows filling of the ventricles with blood before they contract and pump blood to lungs and systemic circulation.

Cardiac muscles constitute most of the cell mass of the heart. In addition to the cardiac muscles and cells involved in conducting electrical impulse, the other types of cells in the heart are: endothelial cells, fibroblasts and connective tissue cells. The generation and propagation of electrical impulse and contraction of muscles is mediated by a variety of membrane transport proteins and channels (such as Ca^{2+}, Na^+, K^+ channels, Na^+-K^+, Na^+-Ca^{2+} transporters, etc.), and several intracellular enzymes and proteins (such as actin, myosin, troponin, esterases, etc.). Many cardiotoxic substances target these macromolecules and prevent their normal function.

The rhythmicity and conduction of the electrical impulse in the heart is regulated by autonomic nervous system (parasympathetic and sympathetic nervous systems), and this system too, can be affected by several toxicants.

Blood vessels: the vascular system is composed of arteries and veins of various diameters (or sizes), and the network of capillaries. The vascular system not only provides a conduit through which blood travels to different organs, but also controls the activity of various organs through production and release of chemical mediators. For example, endothelin produced by endothelial cells modulate cardiac rhythmicity.

Figure 11.1B, provides transverse sections (TS) of a large artery and vein. Observe various tissue layers in the artery and vein. In both the types of vessels, three layers can be identified: the inner most layer 'tunica intima' composed of endothelial layer (a continuous sheet of flat cells) supported with a thin layer of connective tissue. The tunica intima is encircled by a thick layer of smooth muscles called–'tunica media'. The outer most layer 'tunica externa' is made of collagen and elastic fibres. The thickness and composition (such as presence of elastic fibres) of each of these layers differs between arteries and veins, and between arteries of different diameters.

Large arteries such as 'aorta' are rich in elastic fibres (elastic arteries) and the so called 'muscular arteries' (medium sized arteries) are rich in smooth muscles in their tunica media layer. Arterioles are thinner vessels which supply blood to the organs and are mainly composed of a few smooth muscle layers. The diameter of arterioles (thus, the blood pressure) is controlled by autonomic nervous system. The arterioles branch into a network of capillaries composed of tightly placed endothelial cells.

Unlike arteries, which are thick walled wherein blood flows with relatively higher pressure; veins are thinner, have thinner smooth muscle layer, and blood flows through them at a lower pressure. The walls of veins are less elastic and thus can hold large amount of blood. Venules are the smallest thin-walled veins which receive blood from the capillaries.

After being pumped by the heart, blood takes following course:

Heart—aorta—muscular arteries—arterioles—capillaries—venules—veins—back to heart

The endothelial cells and smooth muscles are the main targets sites of toxicants acting on the vascular system.

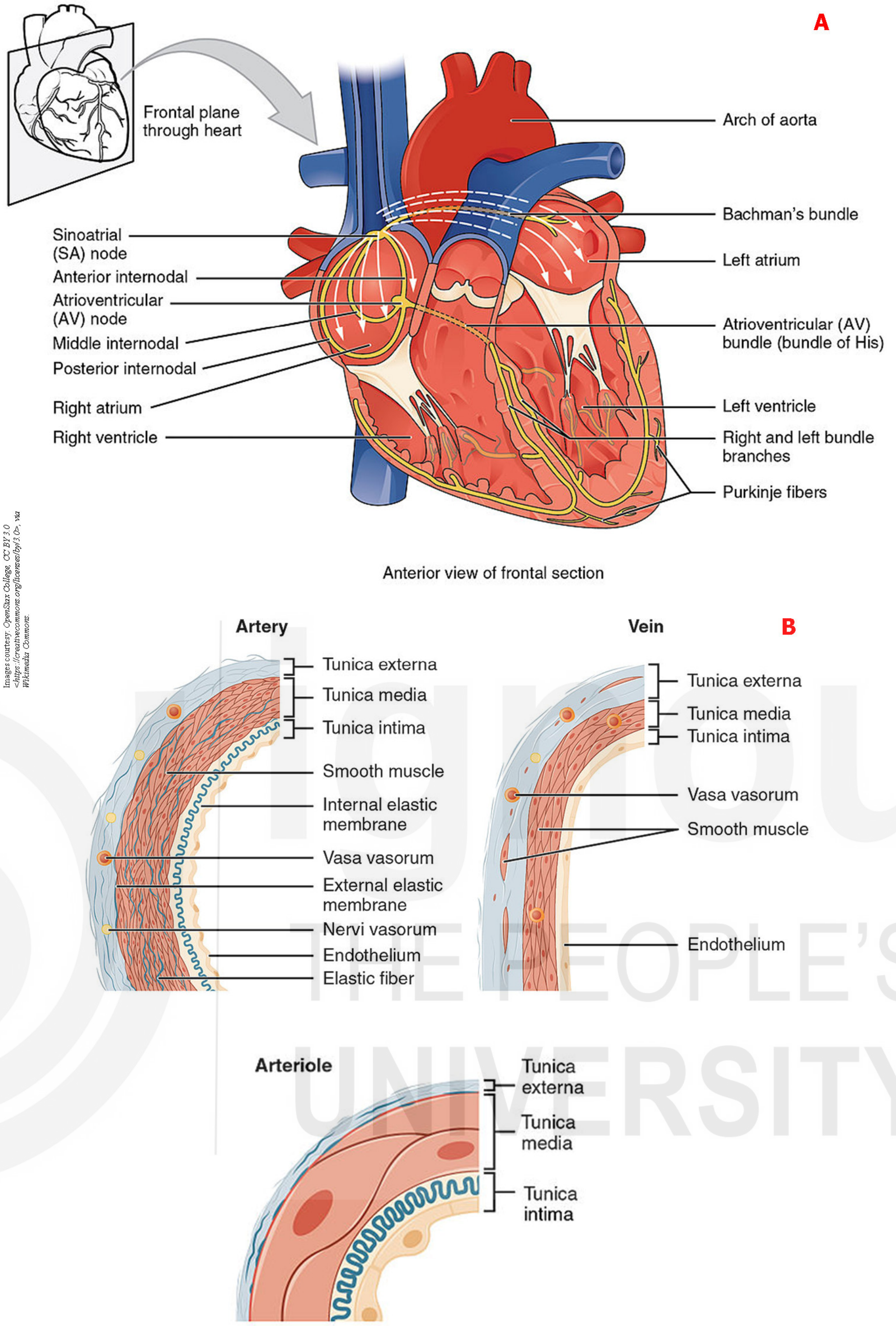

Fig. 11.1: Basic anatomy of heart (A), and blood vessels (B). (Source: OpenStax College, CC BY 3.0 httpscreativecommons.orglicensesby3.0 via Wikimedia. Commons)

11.2.2 Toxic Responses of Cardiovascular System

Various toxicants can interfere with generation and propagation of electrical impulse in the heart, cause injury, and malfunction and death of cardiac muscle cells. Thus, toxicants can alter excitability of cells involved in generation and conduction of electrical impulse, energy metabolism, biochemical pathways, and the contractility and structure of cardiac muscles. Alteration in the function and structure of cardiac muscles by toxicants is known as 'toxicological cardiomyopathy'. Death of cardiac muscles due to chronic or acute toxic insult and the replacement of dead cells with fibres (scar) is the ultimate toxic response. The manifestations of toxic injury to heart are cardiac arrhythmias (slow, fast or irregular heart beat), hypertrophy of cardiac muscles, and heart failure. Acute toxic effects on heart may appear in the form of cardiac arrhythmias and sudden cardiac death, whereas chronic toxic effects can cause cardiac hypertrophy.

The toxic response of vascular system is mainly manifested in the form of changes in blood pressure i.e. hypertension or hypotension, atherosclerosis and edema.

11.2.3 Toxic Agents Acting on Cardiovascular System

Snake venom toxins: the venom of various snakes contains peptides and proteins which have cardiotoxic properties. Let us see a few examples of such toxins:

i. *Bradykinin-potentiating peptides:* These small peptides of 5–14 amino acids inhibit angiotensin-converting enzyme (ACE) which converts angiotensin I to angiotensin II– a potent vasoconstrictor. The toxin therefore lowers blood pressure (hypotension) and reduced cardiac output.

ii. *Sarafotoxins:* These short peptides of 21 amino acids cause vasoconstriction by binding with endothelin receptor. This causes left ventricular dysfunction and cardiac arrest.

iii. *Three-finger toxins:* These 57–82 amino acid long peptides form pores in cardiac muscle cells causing an increase in intracellular calcium ion concentration that activates certain peptidases that disintegrate the cells.

Alcohol: Long term high ethanol consumption causes the condition 'alcoholic cardiomyopathy' which is characterized by dilation of ventricles, increase in the mass of cardiac muscles, and heart failure. The mechanism of alcohol cardiomyopathy is excessive production of acetaldehyde which affects protein synthesis, alteration in mitochondrial respiratory system and contractile machinery of cardiac muscles. Further, generation of free radicals causes lipid peroxidation and, oxidation of membrane proteins.

Cardiac Glycosides*:* These substances are found in plants such as foxglove, milkweed, oleander, etc. Some of the cardiac glycosides such as digoxin and digitoxin are used for the treatment of heart failure. Poisoning due to cardiac glycosides causes death due to ventricular fibrillation (a type of arrhythmia). Cardiac glycosides inhibit Na^+/K^+-ATPase–a transporter that pumps Na^+ out of the cardiac muscle cells which causes increase in Na^+ concentration inside cardiac muscle cells. This causes slowing down of transporter that expels Ca^{++} out of cardiac muscles and thereby retention of Ca^{++} . Accumulation of Na^+ and Ca^{++} in ventricular muscle cells increases automaticity and ventricular arrhythmia.

Doxorubicin: Cardiotoxicity is the major adverse effect of this anti-cancerous drug. High dose of doxorubicin causes ventricular failure and congestive heart failure. Doxorubicin causes generation of free radicals which can cause lipid peroxidation, DNA and protein damage. The final outcome of damage to cellular macromolecules is death of cardiac muscles.

Methylxanthines: Theophylline, caffeine, theobromine, etc. (active components in tea leaves, coffee bean and chocolate) are methylxanthines. Overdosing of theophylline causes ventricular arrhythmias. This is due to the inhibition of phosphodiesterase enzyme and consequent increase of cyclic AMP in cardiac myocytes. High cyclic AMP stimulates release of more calcium from sarcoplasmic reticulum resulting in increase in force of cardiac muscle contraction. In addition, theophylline also increases catecholamine (epinephrine) in blood, which increases the heart rate and force of cardiac muscle contraction.

Heavy metals: Long term exposure to heavy metals such as Cadmium and Lead may cause cardiac hypertrophy and degenerative changes in heart. The toxic effect of these metals is due to their ability to form complexes with proteins and antagonize actions of calcium.

Both lead and cadmium are associated with hypertension. Lead mimics calcium ions, and increases blood pressure by multiple mechanisms including: activation of rennin-angiotensin system, prevention of cGMP-dependent vasorelaxation, and retention of calcium ions inside vascular smooth muscles (by decreasing Na^+/K^+-ATPase activity) which increases the tone of muscle fibres and narrowing of blood vessels.

Cocaine: Cocaine inhibits the membrane transporter protein which removes catecholamine (dopamine, norepinephrine and epinephrine) from the neuronal synapses. This increases the concentration of catecholamine at the synapse resulting in prolonged and enhanced stimulation of sympathetic system and generalized vasoconstriction, increased heart rate and contractility. These conditions may lead to hypertension, chest pain, acute myocardial infarction and arrhythmias.

Tricyclic antidepressants: Overdosing of tricyclic antidepressant drugs causes ventricular arrhythmias and sudden cardiac death. These drugs show toxic effects due to their ability to alter flow of ions (sodium, calcium and potassium ions) across cell membrane of cardiac muscles and Purkinje fibres. In addition, tricyclic antidepressants can also block alpha adrenergic receptors causing hypotension.

General anaesthetics: General anaesthetic drugs such as halothane, isoflurane, etc. reduce contractility of cardiac muscles, cardiac output and mean arterial pressure, and produce arrhythmias. Halothane inhibits voltage gated calcium channels resulting in inadequate concentration of calcium inside the cardiac muscles required for contraction.

SAQ 1

Do as directed:

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

i) Toxicants mimicking actions of catecholamines can increase cardiac muscle contractility and cause hypertension (True/False).

ii) The cardiotoxic actions of doxorubicin are due to the generation of free radicals (True/False).

iii) General anaesthetics such as halothane cause a decrease in cardiac muscle contractility (True/False).

b) Fill in the blanks with appropriate words:

i) Dead cardiac muscles are replaced by...............

ii) Cardiac glycosides are toxic because these can inhibit.........

11.3 NEUROTOXICITY

Nervous system (Central Nervous System or CNS and Peripheral Nervous System or PNS) is a vital system of our body. There are two main cell types found in the nervous system i.e. neurons and glial cells (Schwann cells, oligodendrocytes, astrocytes, etc.). Though nervous system is protected by barriers (Blood Brain Barrier or BBB and Blood Nerve Barrier or BNB), many toxicants can get entry due to their lipid solubility, by using membrane transporter proteins, and from certain sites where BBB and BNB are less effective (such as dorsal root ganglia and nerve terminals).

Toxicants can affect nervous system: i) by damaging neurons and glial cells ii) by interacting with the system of neurotransmission and signalling, causing less or no damage to neurons or glial cells. The latter type of adverse effect is produced by many drugs, drugs of abuse, toxins (such as nicotine, cocaine, glutamate, toxins including tetrodotoxin, botulinum toxins, etc.) and environmental pollutants (such as DDT, mercury), etc. These toxicants can inhibit or excite membrane receptors, transporters and channel proteins (thereby reduce or augment the function of neurons).

In this section, we will discuss about toxicants which harm nervous system by damaging neurons and glial cells.

11.3.1 Susceptibility of Nervous System to Toxic Insult

The main reasons for the susceptibility of nervous system to toxicants include:

i) A constant high energy demand to maintain ionic balance across the cell membrane necessary for generation and conduction of electrical impulse. High-energy requirement makes neurons a target for toxicants which inhibit cellular respiration (e.g. cyanide) or prevent delivery of oxygen (carbon monoxide).

ii) Neurons are large cells, and the metabolites and macromolecules synthesized in the cell body are continuously transported to distant (up to 1 meter) parts of axon and axon terminal (axonal transport). This is achieved by a unique assembly of cytoskeletal elements and motor proteins. The entire process of 'axonal transport' is susceptible to toxicants acting on cytoskeletal and motor proteins.

iii) The function of various ion channels, transporter proteins and membrane receptors, and neurotransmitters involved in transmission of electric signal can be blocked/enhanced by numerous toxicants (see above for examples).

11.3.2 Toxicant Induced Neuronal Damage

Before we discuss neuronal damage caused by toxicants, let us quickly recall the structure of a neuron. Refer to the left most diagram, labelled as 'normal neuron' in Fig. 11. 2 (an illustration of a myelinated multipolar neuron). It has a 'cell body' with four projections representing 'dendrites'. The fifth and longest projection 'axon' is encircled by 'myelin sheath' at regular intervals. The axon terminates at 'axon terminal' which makes synapse with other neurons or effector cells. Myelin is formed by oligodendrocytes in CNS (brain and spinal cord) and by Schwann cells in PNS (nerves). Most of the neuronal metabolism, synthesis of proteins and other metabolites take place in the cell body. The metabolites and proteins are transported via axonal transport system to the distal parts of axon. The cell body is thus crucial for the survival of a neuron.

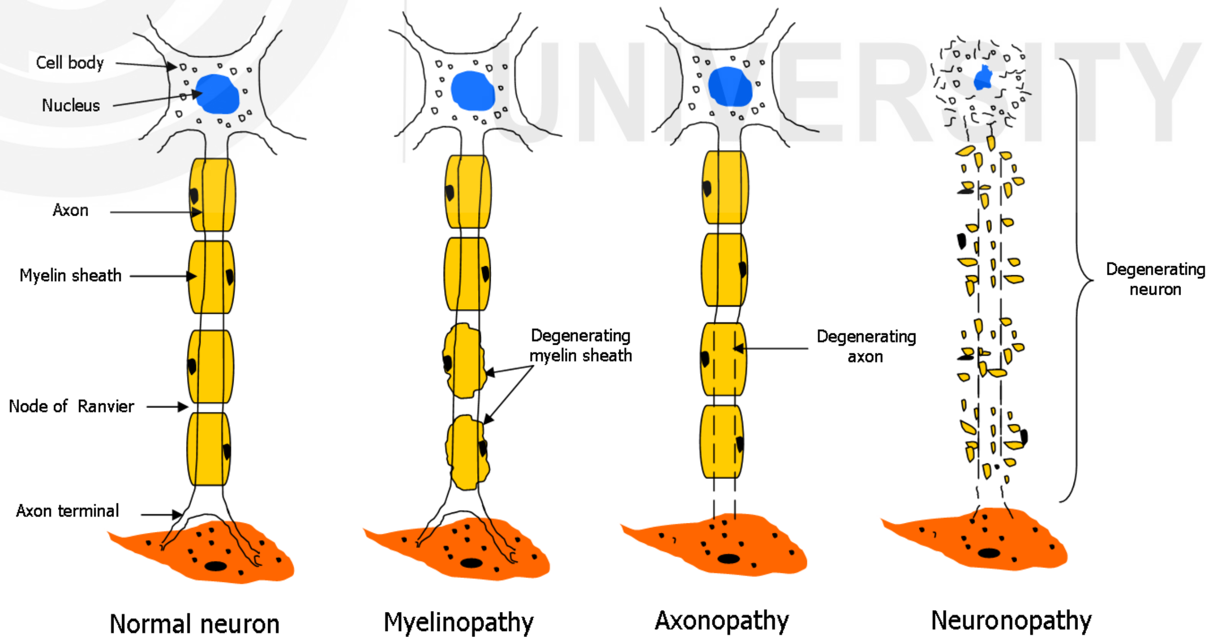

Fig. 11.2: Toxicant induced damage to neurons.

With this basic structure in mind, let us see the structural damages/changes in neurons caused by toxicants.

Again refer to Fig. 11.2, where the following three basic types of neuronal damages have been shown:

i) Neuronopathy

ii) Axonopathy

iii) Myelinopathy

We will briefly discuss each of these below:

i. **Neuronopathy:** In this type of neuronal damage, the primary site of injury is the cell body. Since cell body is the centre for metabolism (see above), a lethal injury to cell body also causes degeneration of axon and dendrites. Thus, neuronopathy is the death of entire neuron with no possibility of regeneration. For example, carbon monoxide and cyanide cause death of neurons in globus pallidus and cortex area of brain. Individuals surviving after poisoning by these two toxicants show Parkinson-like symptoms.

ii. **Axonopathy:** In this type of neuronal damage, the primary site of injury is axon. The part of axon distal to the point where injury is made degenerates together with the myelin sheath. In axonopathy, the cell body remains intact. Toxicants which affect axonal transport system (or cytoskeletal system) cause axonopathy. This is because cytoskeletal system is involved in transport of proteins and other metabolites from cell body to the distal part of axon. In PNS, the surviving Schwann cells and macrophages create an environment which helps in regeneration of axon. Regeneration of axons, however, does not happen in CNS.

iii. **Myelinopathy:** In this type of neuronal damage, the injury is located in cells involved in forming myelin sheath around axon (oligodendrocytes and Schwann cells). The result is demyelination of axons. The axons of PNS can undergo remyelination by Schwann cells. However, the process of remyelination in CNS is not as efficient as in PNS.

11.3.3 Neurotoxic Agents

Let us see a few neurotoxic agents that cause structural damage to neurons.

Methyl Mercury: Humans are exposed to methyl mercury via food. In adults, poisoning of methyl mercury causes damage to visual cortex leading to blindness. In children, exposure to methyl mercury causes developmental abnormalities. Methyl mercury inhibits several crucial cellular metabolic processes including glycolysis, protein and DNA synthesis. It also disrupts electron transport chain and increases the formation of reactive oxygen species (ROS). Further, methyl mercury increases release of glutamate at synapses of neurons which leads to the entry of calcium in adjacent neurons. High calcium levels inside neurons activate cell death pathways and generation of ROS by mitochondria. Cumulatively, all these mechanisms cause death of neurons in CNS.

Doxorubicin: This antibiotic is used for the treatment of cancer. It causes injury to neurons of dorsal root ganglia and autonomic nervous system.

Doxorubicin causes direct DNA damage and overproduction of reactive oxygen species; and affects normal functioning of mitochondria. These lead to degeneration of neurons.

Hexane: Chronic exposure to hexane causes distal axonopathies in longer nerves. The symptoms include tingling and numbness in feet and toes. The condition may progress, where patients cannot walk, stand or hold objects properly. The mechanism of toxicity by hexane includes: conversion of hexane to reactive metabolite '2, 5-hexanedione' by CYP450; reaction of 2, 5-hexanedione with amine groups of proteins (including cytoskeletal proteins) followed by cross-linking of proteins. This leads to impairment of axonal transport system and accompanying degenerative changes in neurons.

Carbon Disulfide: Humans are exposed to this chemical in rubber and rayon production industries. Chronic exposure to carbon disulfide causes peripheral neuropathy and neuro- physiological changes. It causes distal axonopathy by cross-linking cytoskeletal proteins. Carbon disulfide reacts with amine groups of proteins and derivatizes it to reactive isothiocyanate group. The latter reacts and cross-links with thiols and amine groups of other proteins.

Colchicine and Vinca alkaloids: Colchicine is used for treatment of gout, it prevents depolymerisation of microtubules. Vinca alkaloids (vincristine and vinblastine) are used for the treatment of cancer. These alkaloids bind to tubulin (a cytoskeletal protein) and prevent its assembly into microtubules. Both the alkaloids thus affect axonal transport system of neurons leading to degeneration of axons in PNS.

Tellurium: This element is used in rubber, copper and electronic industries. In PNS, it causes demyelination of axons of sciatic nerve. Demyelination is followed by remyelination of neurons and restoration of normal function. The mechanism of demyelination by tellurium includes damage to Schwann cells, by preventing biosynthesis of cholesterol– an important component of myelin. Lack of cholesterol destabilizes and damages the myelin sheath.

Hexachlorophene: It is added to cosmetics (soaps, deodorants, body powders, lotions, etc.) and oral hygiene products (mouthwashes, toothpastes, etc.) as preservative. It causes splitting of myelin layers in CNS and PNS. The mechanism includes: binding to the cell membrane and prevention of removal of ions between layers of myelin sheath; the high ion concentration between layers of myelin sheath attracts water resulting in the separation of individual myelin layers. With continuing exposure to hexachlorophene, there is demyelination of axons and swelling of brain which can be fatal.

SAQ 2

Do as directed:

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

i) Nervous system is susceptible to toxic insults because its constituent cells i.e. neurons require high energy for proper functioning (True/False).

ii) Axons in peripheral nervous system can regenerate after degeneration due to toxic insult (True/False).

iii) In neuronopathy, only cell body of a neuron is damaged whereas axon remains intact and functional (True/False).

iv) Tellurium, a neurotoxicant causes damage to glial cells of CNS (True/False).

v) Hexane shows toxic effect in nervous system because its metabolites can crosslink lipids of cell membrane (True/False).

b) Fill in the blanks with appropriate words:

i) The process in which only axon of a neuron is damaged is called.............

ii) Colchicine shows toxic effect because it can inhibit.................. of microtubules

iii) The cell responsible for myelination in CNS is......................

11.4 SUMMARY

- The cardiovascular system is composed of heart and blood vessels (arteries, veins and capillaries). Heart has two main types of tissues– cardiac muscles and cells involved in generation and propagation of impulse. The cells of both the tissues are highly active and require large amount of energy for continuous pumping of blood. The cell membranes of these cells have varieties of transporters and ion channels required for maintaining proper gradient of ions necessary to generate and propagate impulse or action potential. The cytoplasm contains varieties of enzymes and proteins involved in processing of signals and contraction of cardiac muscles. All the above-mentioned features make heart susceptible to toxic insult.

- Structurally, the arteries and veins are composed of three layers: the inner layer of endothelial cells, the middle smooth muscle layer and the outer connective tissue layer. The most important function of vascular system is to deliver blood to various organs to fulfil their requirement of nutrients and oxygen. Endothelial cells and smooth muscle cells are the main target sites of toxicants in blood vessels.

- The toxic manifestations of heart can be in the form of cardiac arrhythmias, hypertrophy of cardiac muscles, particularly the ventricles; and in extreme cases, heart failure. Toxicants can cause injury to cardiac muscles by several mechanisms. The cardiac muscles cells died due to toxic insult are replaced by non-functional tissue–the fibrous tissue, with a compromise in the function of heart.

- Alteration in the blood pressure and thickening of blood vessels or fatty depositions in the walls of blood vessels are the main toxic manifestations of vascular system.

- Of the two main cell types of nervous system i.e. neurons and glial cells, the former is more susceptible to toxic insult. The features of neurons which make them susceptible include high energy requirement, presence of various membrane transport proteins and inability of nervous system to replace lost neurons.
- Toxicants can impair functioning of neurons by affecting membrane transport systems, synthesis, release and removal of neurotransmitters. These toxic responses however, are not accompanied by any major structural changes in the nervous system or damage to neurons.
- In the second type of toxic response, there is damage to neurons or glial cells by toxic agents. A total loss of neurons due to toxic insult is termed as 'neuronopathy'. Neuronopathy causes irreversible loss of neurons since the latter cannot regenerate. Some toxic agents (e.g. colchicine) can cause damage to axon– a process known as axonopathy, whereas others (e.g. tellurium) can demyelinate axons. In PNS, the latter two types of neuronal damages can be regenerated or repaired.

11.5 TERMINAL QUESTIONS

1. Provide examples of two cardiovascular toxicants with their mechanisms of toxicity.
2. What is Neuronopathy? Provide example of one toxic agent which causes Neuronopathy.
3. Neurons are susceptible to toxic insult. Discuss.

11.6 ANSWERS

Self-Assessment Questions

1. a) i) True ii) True iii) True

   b) i) Collagen fibres ii) Na^+/K^+-ATPase

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

   b) i) Axonopathy

   ii) Depolymerisation

   iii) Oligodendrocytes

Terminal Questions

1. *Cardiac Glycosides:* It inhibits Na^+/K^+-ATPase, a transporter involved in export of sodium ions from inside to outside of the cell. Inhibition of this transporter increases Na^+ concentration in the cardiac muscles. This slows down function of another membrane transport protein which expels Ca^{++} (calcium is necessary for muscle contraction) out of the cell. Accumulation of Na^+ and Ca^{++} causes ventricular arrhythmia. In severe poisoning cases, a victim may die due to ventricular fibrillation (a type of arrhythmia).

Doxorubicin: A high dose of doxorubicin causes ventricular and congestive heart failure. Doxorubicin causes generation of free radicals which can cause lipid peroxidation, DNA and protein damage. The final outcome of damage to cellular macromolecules is death of cardiac muscles.

2. It is a type of neuronal damage in which toxicants cause lethal injury to the cell body of neurons. Since cell body is the centre of metabolism, death of cell body causes degeneration of axon and dendrites as well. Thus, neuronopathy is death of entire neuron.

   Example: methyl mercury, which inhibits several crucial cellular metabolic processes including glycolysis, protein and DNA synthesis, and causes increases in the formation of reactive oxygen species (ROS). Methyl mercury also increases calcium levels in neurons. A high calcium levels inside neurons activate cell death pathways and generation of more ROS by mitochondria.

3. Neurons have a constant demand of energy for generation and conduction of nerve impulse. Neurons are dependent on aerobic respiration to get energy, therefore, toxicants inhibiting mitochondrial respiration or ability to transport oxygen to brain can cause neuronal injury. Neurons have a high number of various transporters and ion channels, which mediate proper ion gradient necessary for generation of impulse and its conduction. These channels are potential molecular target sites for a number of toxicants.

11.7 FURTHER READINGS

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