

Unit 10

NEPHROTOXICITY |

Structure

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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. In this 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 *papillar* receives 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 cells of 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.

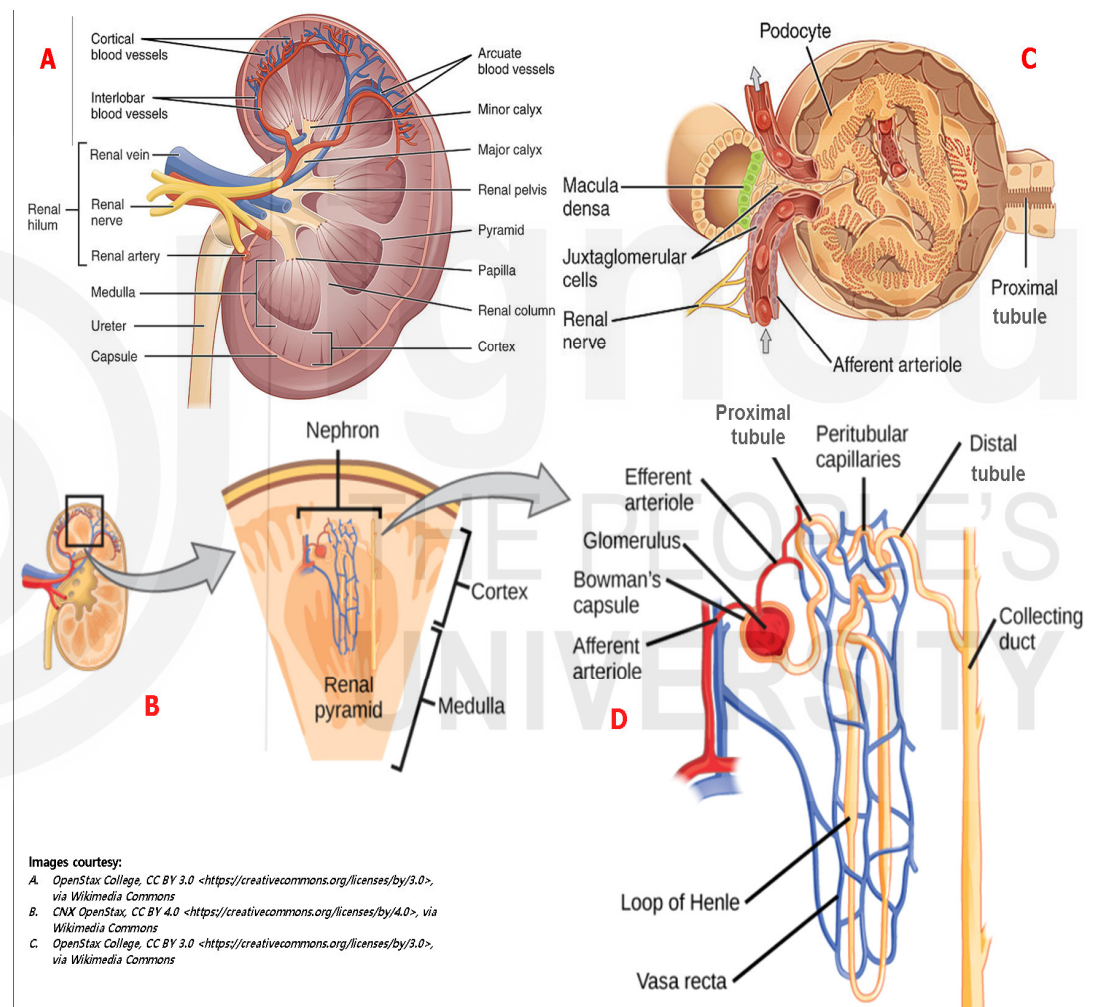


Fig. 10.1: Functional anatomy of kidney; (A) gross anatomy showing various parts of kidney; (B) showing position of nephrons in kidney; (C) a glomerulus showing glomerular capillaries covered intimately by podocytes and; (D) various parts of a nephron and blood supply to the nephron.

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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 andare 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 as prolonged 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 nephrons 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.

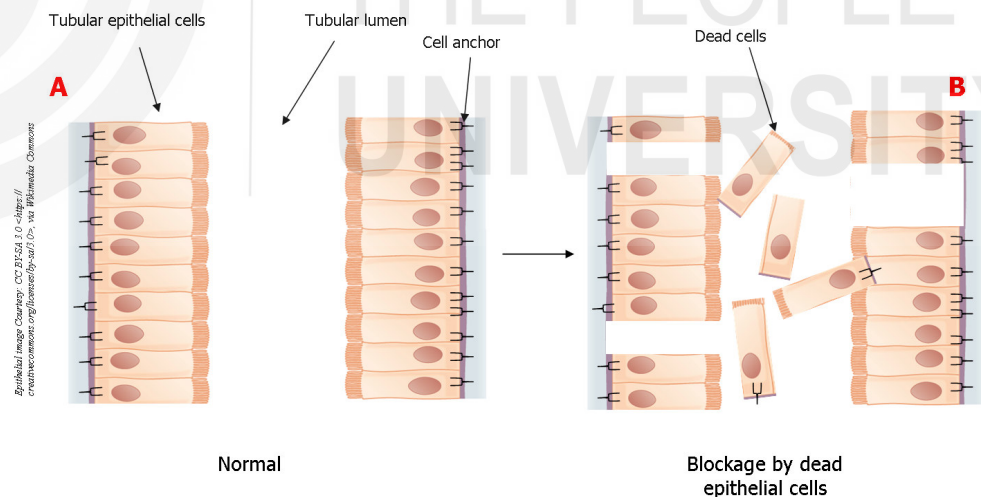


Fig. 10.2 : Cell death in the tubular lumen of nephron; (A) normal tubular lumen and; (B) dead cell debris blocking the tubular lumen.

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).

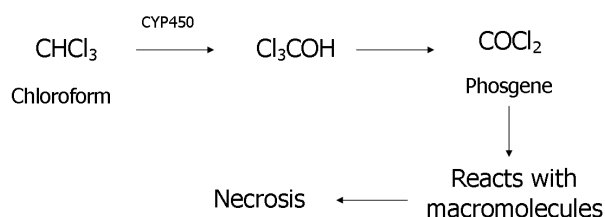


Fig. 10.3: Mechanism of nephrotoxicity by chloroform.

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 tubules leading 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).

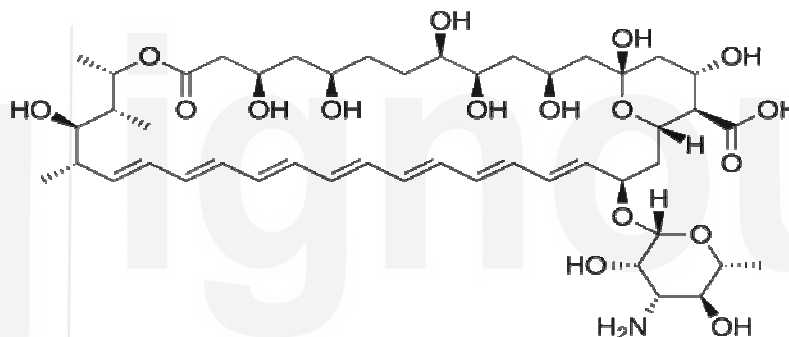


Fig.10.4: Amphotericin B.

(Source: The chemists, 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 causes necrosis and tubular atrophy due to oxidative stress (Fig. 10.5).

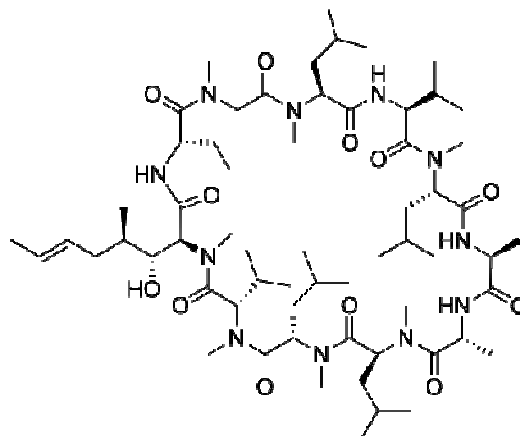


Fig.10.5: Cyclosporine.

(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), $\text{CH}_3\text{-Hg}$ (organic mercury) and Hg^0 (elemental mercury). Elemental mercury is converted to inorganic mercury in the body, and the latter form conjugates with the $-\text{SH}$ (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.

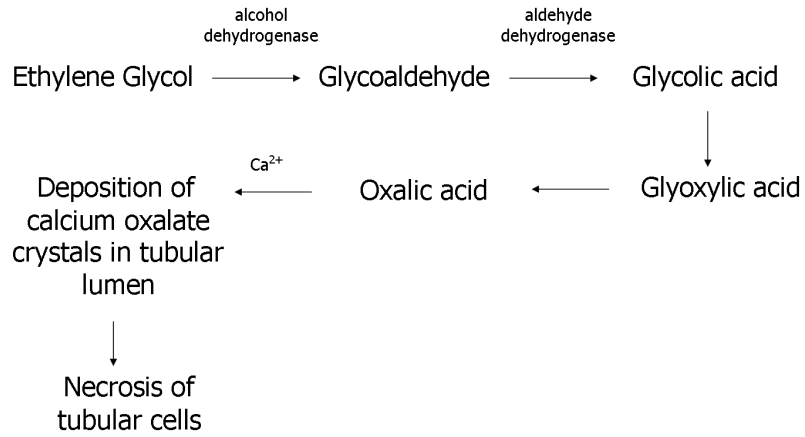


Fig.10.6: Metabolism of ethylene glycol to oxalic acid and resultant injury to kidneys.

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.
- Major nephrotoxicants 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.

10.8 FURTHER READINGS

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