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TOXICOLOGY
NEWSLETTERS

John Putnam Merrill *(March 10, 1917 – April 14, 1984)* was *an American physician and medical researcher. He led the team which performed the world's first successful kidney transplant. He generally credited as the "father of nephrology" or "the founder of nephrology"*

Dr. John P. Merrill (left) explains the workings of a then-new machine called an artificial kidney to Richard Herrick (middle) and his brother Ronald (right). The Herrick twin brothers were the subject of the world's first successful kidney transplant, Ronald being the donor.

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Nephrotoxicity

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1. Preamble

Over the last 20 years, it has become increasingly obvious that the kidney is adversely affected by an array of chemicals. Humans are exposed to these as medicines, industrial and environmental chemicals, and a variety of naturally occurring substances. The level of exposure varies from minute quantities to very high doses. Exposure may be over a long period of time or limited to a single event, and it may be due to a single substance or to multiple chemicals. *The circumstances of exposure may be inadvertent, accidental, or intentional overdose or therapeutic necessity. Some chemicals cause an acute injury and others produce chronic renal changes that may lead to end-stage renal failure and renal malignancies.* These include those nephropathies caused by cadmium, other environmental heavy metals, and, more recently, the organo-metallic compounds used as therapeutic agents, anti-cancer drugs, cyclosporin, analgesic abuse, and antibiotics.

The process of inventing drugs and developing through various evaluation endpoints is complex, expensive, time-consuming and herculean task. *Approximately 30% of total novel candidates developed is terminated due to lack of ability to predict adverse effects in animals or human. Hence the pharmaceutical industries try to detect toxic drug effects at the earliest to avoid the progress of such drug candidates into more expensive drug development stages*. Kidney safety assessment in preclinical species is mandatory in novel drug or chemical development. Drug-induced nephrotoxicity is one of the reasons for the failure of selection of candidate to next level of its testing.

Owing to kidney's diverse functions and small mass in relation to the resting

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cardiac output that it handles, the kidney is a target both for chemicals that are pharmacologically active and for toxic material. The nephron and its related cells perform a diversity of physiological functions. It is the major organ of excretion and homeostasis for water-soluble molecules; because it is a metabolically active organ, it can concentrate certain substances actively. In addition, its cells have the potential to bio-convert chemicals and metabolically activate a variety of compounds. Specific physiological characteristics are localized to specific cell types. This makes them susceptible to, and the target for, chemicals. The effect of any chemical on a cell may be pharmacological, in which case the effect is dose-related and occurs only as long as the concentration of the effector is high enough to be active. Alternatively, the chemical may cause damage to the cell. The cell responds to injury by repair and the kidney responds to the cellular lesion by renal and extra renal adaptation to compensate for the loss of that cell function. Although, there is a substantial capacity within the kidney for repair, there are also several circumstances where damage may be irreversible. *In general, the proximal and distal tubules and urothelia can be repaired, but the glomeruli and medulla may have a significantly lower repair facility.* It is, therefore, possible to initiate a series of degenerative changes as a result of interfering with one or more of the normal physiological processes.

2. Kidney Structure and Function

An in-depth review of kidney structure and function is beyond the scope of this newsletter. Only sufficient anatomical information will be given to provide a general background against which nephrotoxicity can be framed. Each kidney is made up of a large number of nephrons, groups of which unite to continue as collecting ducts or tubules, and these, in turn, combine to make up the ducts of Bellini, which exit around the papilla tip. The papilla opens into the calyx, which is in continuity with the renal pelvis, a funnel-shaped area that narrows to the ureter.

A typical kidney contains about a million nephrons that work in tandem to ensure its primary functions, which include filtering waste from the blood, maintaining the overall fluid balance of the body, maintaining blood pH, and hormonal functions that promote red blood cell production, bone health and regulation of blood pressure. Loss of enough cells along any part of the nephron can alter any of these functions. This is particularly true of the proximal tubules, which are the primary target of a vast majority of nephrotoxicants.

a. The Nephron

The kidney is divided into three main regions, cortex (outer), medulla (inner), and pelvis. Within the cortex arise the renal corpuscles, defined as superficial, mid-cortical or juxtamedullary depending on the anatomical location of the renal corpuscle in the cortex. *The nephron is the functional unit of the kidney and consists of a continuous tube of highly specialized heterogeneous cells, which show sub-specialization along the length of nephrons and between them. There are marked structural and functional differences between the nephrons arising in the cortex and those arising in the juxtamedullary regions.* The total number of nephrons varies between different species and within any one species as a function of age.

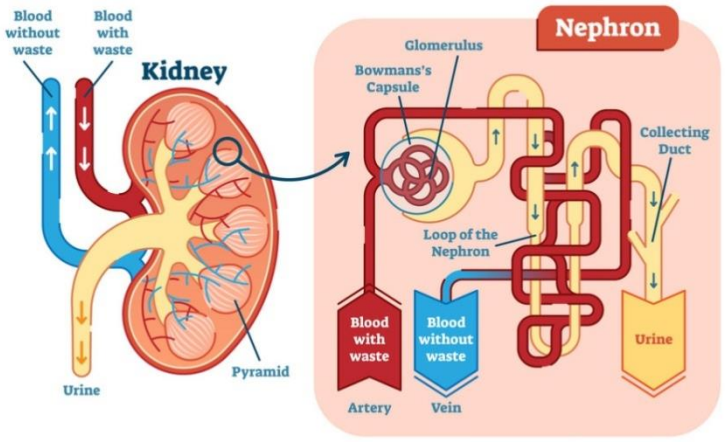

Figure 1 Nephron Anatomy

b. Cellular Heterogeneity and Cell-Cell Interaction

There are well over 20 morphologically different cell types in the kidney. The spectrum of biochemical (and structural and functional)characteristics in these cells demonstrates the very marked heterogeneity that is the hallmark of the kidney. It is well established that the expression of many of these biochemical characteristics is an integral of the functions of that particular region of the kidney, and there is the potential to change the expression of these characteristics in terms of the demands on the kidney. These include both water and electrolytes, dietary factors, and chemicals with pharmacological and toxic effects, or maybe as a result of chemical and other types of injury. *More importantly, the characteristics of a cell may make it either resistant or sensitive to the target-selective toxicity of a chemical.*

c. The Glomerulus

The glomerulus forms the initial part of the nephron and functions as a relatively poorly selective macro-molecular exclusion filter to the hydrostatic pressure of the blood. *The glomerular "tuft" is made up of a number of capillary branches that arise from the afferent arteriole, anastomose, and drain to the efferent arteriole.* The capillaries are in direct contact with the glomerular basement membrane. *The driving force for filtration is provided by the glomerular capillary hydrostatic pressure, minus both the plasma osmotic pressure and the hydrostatic pressure in the Bowman's space.*Selective filtration is achieved primarily on the basis of size restriction by the basement membrane, which impedes the passage of macromolecules with an effective radius greater than 1.8 nm and completely prevents the filtration of macromolecules with an effective radius greater than 4.5nm. *In addition, the presence of fixed negative charges on the endothelial, epithelia, and basement membranes hinders the filtration of anionic macromolecules while facilitating the passage of cationic macromolecules.* The selectivity of filtration is, in part, a consequence of the anionic nature of the basement membrane, which blocks or slows the passage of negatively charged or neutral macromolecules and leaves those carrying a cationic charge and small molecules to pass unimpeded.

d. The Proximal Tubule

The proximal tubule is found only in the cortex or sub cortical zones of the kidney. Anatomically, each proximal tubule can be divided into the convoluted portion and the shorter straight descending portion, which then continues to become the descending limb of the loop of Henle. *The proximal tubule plays a decisive role in maintaining homeostasis. This is achieved when sodium and chloride ions flux from the tubule lumen to the peritubular capillaries under the control of a number of processes such as nonspecific electrophysiological gradients and selective active transport mechanisms.* Water follows the ions by osmotic effects. In addition, hydrostatic pressure, attributable to the presence of both proteins and glycosaminoglycans, contributes to water movement from the epithelial cell to the interstitium and thence, by an osmotic gradient, into the capillaries. The flux of ions within the proximal tubule, including the absorption and secretion of HCO3- and H+ and the "lumen trapping" of ammonium ions, controls renal acid-base regulation. Those proteins that have passed from Bowman's capsule (a significant amount of albumin in the case of normal rats) are reabsorbed in the proximal tubule by pinocytotic removal from the base of the microvillous brush border into the epithelial cells. The vesicles thus formed combine, form protein-filled vacuoles, and fuse with lysosomes, from which the digestion products of the protein diffuse, eventually, to the capillary system or are used in the metabolic processes of the cell.

e. The Medulla

The medulla can be divided into the outer medulla and the inner medulla, the free part of which is referred to as the "papilla". The inner medulla contains the thin limbs of the loops of Henle, collecting ducts, the vasa recta, and a diffuse network of capillaries. Packed into the spaces between these structures are interstitial cells embedded in a matrix rich in glycosaminoglycans. *The collecting ducts terminate as the ducts of Bellini around the tip of the papilla. Whereas the mouse, gerbil, rat, guinea-pig, rabbit, dog, cat, and primate kidneys have only a single papilla, the pig and man have multi-papillate kidneys.*

f. The Loops of Henle

The loops of Henle may be divided into short loops which penetrate no further than the outer medulla. The proximal tubule and thick ascending limb are closely associated in the cortex, but in the medulla, the descending limb is intimately related to the ascending vasa recta and the ascending limb to the collecting duct. The Loop of Henle provides a shunt that excludes selected solutes (and water)from the inner medulla. *This exclusion of water and trapping of sodium chloride, urea, and osmolytes helps maintain the osmotic gradient along the inner medulla.*

g. Collecting Ducts

Collecting ducts consists of three identifiable segments, which lie, respectively, in the cortex, the outer medulla, and the innermedulla. *These segments demonstrate different permeabilities to water and osmolytes.* The difference in permeability may be related to the presence of two cell types, the intercalated and collecting

duct.

h. The Distal Tubule

The distal tubule connects the thick ascending limb of the loop of Henle to that part of the collecting duct which originates in the cortex. The distal tubules are involved in both ion and water reabsorption but plays a much less significant role than the proximal tubules. *The major differences include a stronger Na+ gradient against which to "pump", the ability to reabsorb sodium without reabsorbing water, the controlling effects of anti-diuretic hormone (ADH) and aldosterone, and the very limited protein reabsorption.*

3. The Metabolism of Xenobiotic Molecules In The Kidney

Chemically induced lesions may depend to varying extents on the metabolic capacity of tissues to deal with "insults". The metabolism of xenobiotic molecules may either prevent lesions (by deactivation) or be directly responsible for damage (by bio-activation). Kidney microsomes have been shown to have most of the enzymic and cytochrome-mediated metabolic activities that have been described in other tissues. *The xenobiotic-transformation capacity of the kidney is about 3-50% (depending on the system, species, and source of data) of that found in the liver.*

There are several enzymes involved in renal xenobiotic metabolism. It is not possible to comment on all of those that maybe relevant to nephrotoxicity nor, indeed, is it clearly established what role each renal enzyme plays in the realization of the potential toxicity of a chemical. Many of the enzymes that metabolize xenobiotics are compartmentalized in specific regions of the kidney. The anatomical localization of these characteristics may play a key role in the toxicological consequence that follows the entry of a xenobiotic into the kidney. *The intracellular concentration of a chemical can be influenced by xenobiotic metabolism per se and by many of the inherent processes in the kidney, such as transport, pH, and solute gradients on either side of a membrane.*

A. Oxidases

Oxidases can convert chemicals into active intermediates or generate reactive species by redox cycling. This is potentially important for compounds that contain arylamine, quaternary bipyridyl (paraquat), quinone (adriamycin), or nitro (nitrofurantoin) structures. Biologically reactive intermediates mediate their toxic effects by binding to cellular macromolecules and blocking normal functional processes. *The metabolically generate reactive intermediates have a relatively short life and are most likely formed in the organ or anatomical area in which they induce damage.*

B. Cytochrome-P-450-Dependent Mixed-Function Oxidases (Monooxygenases)

This enzyme system carries out a two-electron flow pathway, and the flavoprotein component can catalyse single electron reductions such as the reduction of quinones to semiquinone radicals. Multiple forms of cytochrome P-450 have been identified in the kidney. The specific activity of the renal mixed-function oxidases varies widely between species and is about 10% of the hepatic

activity. This suggests a role for renalP-450 that is quantitatively less important than that of the liver. However, this is not the case for all chemicals, since the renal metabolism of chloroform is about 2-fold higher than the hepatic activity. *The S2 proximal segment has a cytochrome P-450 concentration that is 2-3 times higher than the S1 or S3 segments. The distal tubules, cortical collecting ducts, and the medulla contain no measurable cytochrome P-450 activity.* By contrastNADPH-cytochrome-P-450 reductase activity is highest in the S2 andS3 segments, but it also extends to the distal tubule and medullary structures.

C. Conjugation

Conjugation takes place on existing groups or those produced by oxidation and greatly increases the polarity of compounds. This facilitates their elimination and generally terminates any pharmacological activity. There are several examples, however, where conjugation may give rise to reactive compounds *(e.g., the glucuronides of N-hydroxy-2-acetylaminofluorene and N-hydroxyphenacetin are potently toxic). Similarly, glutathione conjugates may be toxic.*

a. Glucuronide Conjugation

Glucuronide conjugates are formed by the action of uridinediphosphate (UDP) glucuronyl transferase. This enzyme has at least three isozymes, each of which preferentially conjugates different types of molecules. *Only UDP-GT1 occurs in the rat kidney, where the substrates include planar compounds such as 1-naphthol and4-nitrophenol. UDP-GT1 activity is increased by 3-methylcholanthrene.*

Human kidneys have UDP-GT1 and high GT2 activities, *while rabbit kidneys have all three isoenzymes.* UDPglucuronyl transferase activityis highest in the cortex, and the distal tubule activity is about 50%of that found in the proximal tubule. Renal glucuronidation capacity may be comparable to or greater than that of the liver, depending on the substrate, and microsomes from female rats form considerably *more glucuronide conjugates than those from male rat kidneys.*

b. Sulfate Conjugation

Sulfotransferases form highly polar and, therefore, rapidly excreted sulfate esters. *The concentrations of both sulfotransferase and activated sulfate are higher in the renal cortex than in the medulla, and renal sulfotransferase activity is markedly lower than that of the liver.*

c. Glutathione Conjugation

Glutathione is the most abundant thiol-containing peptide in the kidney, where it is synthesized in the proximal tubule and provides a scavenger for detoxifying electrophilic radicals formed from alkyl andaryl halides, epoxides, and alkenes. These compounds are degraded to the cysteine conjugate and are generally excreted as the N-acetyl-cysteine conjugate. Glutathione S-transferase plays an active role in metabolism, where it catalyses the initial step in glutathione conjugation of halogenated aromatics, epoxides, halogenated alkyls and aralkyls, and alpha, ß-unsaturated compounds, and drugs such as paracetamol (acetaminophen), and endogenous substrates such as estrogen and prostaglandins. *The total renal GSH S-transferase activity per g wet tissue is considerably less*

than the corresponding hepatic activity. There are sex differences in the renal GSHS-transferase activities in rats, aralkyl, epoxide, and alkyl transferase activities being lower in males than in females.

d. MercapturicAcid Synthesis

The formation of glutathione conjugates is the first in thepathway of renal metabolism to mercapturic acid. *The enzyme gamma-glutamyl transpeptidase is localized on the brush border of the proximal tubule, where it cleaves the gamma-glutamyl linkage of glutathione to produce the cysteinyl-glycine conjugate in the tubule lumen.* This metabolite is a substrate for a number of peptidases that produce the cysteinyl conjugate, which in turn is converted to the mercapturic acid by microsomal N-acetyltransferase. The cells of the proximal tubule in the outer medulla producethe N-acetyl-cysteine conjugate of paracetamol.

Glutathione conjugation is generally a detoxification pathway,but some compounds may undergo bioactivation by ß-lyase.

e. Amino Acid Conjugation

The amino acid glycine forms a glycoconjugate via the activation of a carboxylic acid (e.g., salicylic acid) by coenzyme A. Salicyl-CoA and glycine are co-substrates for acyl-CoA-glycine- N-acetyltransferase, which catalyses the condensation tosalicyluric acid. Glycine N-acetyltransferase activity is present in the kidneys of rabbits, monkeys, and humans.

The kidney is a major site for the metabolism of benzoic acid to hippuric acid, p-aminobenzoic acid to p-aminohippuric acid, and salicylate to salicyluric acid. The freshly deconjugated salicylate is more rapidly excreted than the parent salicylate. Possibly the diffusion of salicylate into the renal cell is the rate-limiting step of elimination. This is not the case for salicyluric acid, which is converted to salicylate within tubular cells.

4. The Mechanistic Basis of Chemically Induced Renal Injury

There has been a growing understanding of the molecular basis of disease and the biochemical mechanisms thatare associated with chemically induced cellular degeneration and lesions in target organ systems. The application of this understanding provides a foundation upon which to study chemically induced renal injury and, in particular, a rational basis for the extrapolation of animal toxicity data to man and risk assessment.

The two kidneys process 25% of the resting cardiac output via an arterial blood supply. Much of the fluid and most of the solutes in blood are filtered through the glomeruli into the proximal part of the nephron (the functional unit of the kidney) from which essential small molecules are reabsorbed. Numerous macro-molecules are reabsorbed into the tubular cells by an endocytotic process and are digested in tubular lysosomes. *Many organic acids and bases (including many drugs) are secreted (and reabsorbed) by carrier-mediated processes located principally in the proximal tubule.* There is some secretion, mainly of waste solutes, from the blood into the distal part of the nephron, and much of the water

in which they are dissolved is subsequently reabsorbed.

Renal lesions occur in discrete anatomical regions. This highlights the need to understand changes in terms of the biochemical properties of the specifically affected region and its adjacent cells. While haematoxylin and eosin staining and a number of other routinely used staining procedures identify nephropathies and renal degeneration, these are generally based on a relatively non-specific assessment.

5. Nephrotoxicity

Nephrotoxicity can be defined as the adverse effect of substances on renal function. Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants. Exposure to drugs often results in toxicity in the kidney which represents the major control system maintaining homeostasis of body and thus is especially susceptible to xenobiotics. Understanding the toxic mechanisms for nephrotoxicity provides useful information on the development of drugs with therapeutic benefits with reduced side effects. Mechanisms for drug-induced nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. Nephrotoxicants can include molds and fungi, cancer therapeutics such as cisplatin, antibiotics such as aminoglycosides, metals such as mercury, arsenic and lead, and drugs of abuse such as cocaine. *One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or urine output; however, nephrotoxicants can induce kidney damage without changing any established clinical marker of renal function. For example, studies have shown that proximal tubule necrosis in male Sprague Dawley rats exposed to gentamicin can be as high as 75% prior to any increases in BUN or sCr.*

Common features of nephrotoxicity can involve renal cell death including changes in the structure of the functional unit of the kidney - the nephron. This includes changes to the tubules, glomeruli, the interstitium, and the intra-renal blood vessels.

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines, and industrial or environmental chemicals. It is well established that toxic nephropathies are not restricted to a single type of renal injury. Some chemicals target one discrete anatomical region of the kidney and may affect only one cell type. Chemical insult to the kidney may result in a spectrum of nephropathies that are indistinguishable from those that do not have a chemical etiology.

6. Renal Functional Reserve

The concept of the renal functional reserve is a simple one in which not all the nephrons nor all of the cellular functions in a single nephron is available or used at any one time. Thus, there is a buffering capacity in the kidney that can cope with short-lived or protracted demands on a function that exceed the normal level.

Part of this functional reserve is used to meet the response to perturbation of the homeostatic system by water or electrolyte loading. Most of the studies on an understanding of renal functional reserve relate to changes in glomerular filtration rate (GFR) and renal blood flow.

7. Biomarkers For Assessment of Nephrotoxicity

The most efficient way to prevent or mitigate nephrotoxicity is to have sensitive and specific biomarkers that can be used in animals early in drug development, well before clinical studies are underway. *The reasons for drug-related kidney damage could be related to on target (pharmacological action), off target, metabolism or final pathway of excretion etc.* The detection of kidney damage is difficult with traditional markers [like serum blood urea nitrogen (BUN) and creatinine (SCr)] which are not sensitive and specific as their level increases when significant injury (~30%) to kidney or ~65% of the nephrons lose their functional capacity. This has an impact on adversity assessment in animal toxicology studies resulting potential failure of drugs during the clinical trial. Similarly, the delayed diagnosis of renal disease will result in poor treatment management, or initiation of treatment. Drugs showed to cause toxic effects by several common pathogenesis affecting specific or multiple sites of nephron. Hence, it is important to predict the nephrotoxicity at the earliest stage of nephron damage, which is an unmet need. Over recent years, extensive effort has been directed towards the invention and validation of novel renal safety biomarkers that could be used to predict and monitor drug-associated nephrotoxicity.

These biomarkers should be able to sensitively predict toxicity in preclinical models and clinical situations so that they can be used to efficiently guide drug developers to modify or discard the potential therapeutics and replace them with variants that affect the same target without the toxicity.

Biomarker candidates have been identified for the assessment of nephrotoxicity. Although some of them fail to confer specificity and sensitivity of biomarkers, several promising candidates have been proved for diagnosis of nephrotoxicity. List of biomarkers for nephrotoxicity assessment and drugs in different nephron segments are presented below:

Table 1 List of biomarkers for evaluation of nephrotoxicity

Nephron segment	Drugs inducing nephrotoxicity	Biomarkers
Glomerulus	ACE inhibitor, ARB, NSAIDs, Mitomycin-C	Proteinuria
	Antiplatelet agents, Cyclosporin, Quinone	Albumin, Transferrin, Immunoglobulin G, β2-microglobulin, α1-microglobulin, Cystatin C Retinol binding protein
		Cytokines

		Interferons, Interleukins, TNF, CSFs
		Type IV collagen
Proximal tubule	Aminoglycoside antibiotics	Urinary proteins with enzymatic activity α-GST, N-Acetyl-D-Glucosaminidase Proteinuria
	Amphotericin B, Adefovir	
	Cisplatin, Foscarnet	
	Contrast stain, Cocaine, Heroin, Methadone	Albumin, Transfferin, Immunoglobulin G, β2-microglobulin, α1-microglobulin, Cystatin C Retinol binding protein Cytokines
	Methamphetamine	
		Interferons, Interleukins, TNF, CSFs
		KIM-1, NGAL, Clusterin, Osteopontin
Distal tubule	Amphotericin B, Lithium	NGAL, Clusterin, Osteopontin
	Acyclovir, Indinavir, Sulfonamides	

a. Urinary Proteins with Enzymatic Activity

If there is acute or chronic kidney damage due to exposure to nephrotoxic substances, diabetic kidney disease, hypertension, renal ischemia, transplant, or glomerular diseases, the enzymes present in tubular epithelial cells are spilled into the urine and can be detected as nephrotoxic biomarkers. *Biomarkers related with urinary proteins with enzymatic activity include alanine aminopeptidase, alkaline phosphatase, α-glutathione-S-transferase, γ-glutamyltranspeptidase, π-glutathione-S-transferase, and N-acetyl-D-glucosaminidase.*

b. Proteinuria

In normal conditions, the glomerulus restricts the migration of high molecular weight proteins from blood to nephron lumen by filtration. In some pathological states, however, high molecular weight proteins can be detected in the urine because the selective penetration through glomerulus is not functioning properly. *High molecular weight proteins that can reveal kidney damage include albumin which can be used for early diagnosis of changed glomerular filtration and diabetes, transferrin which transports iron and represents glomerular damage*

more sensitively, and immunoglobulin G that shows structural damage in the glomerulus.

Low molecular weight proteins produced in other organs are filtered and reabsorbed in the glomerulus and not released from the proximal tubule. An increase of the filtered low molecular weight proteins represents that absorption in the glomerulus and proximal tubule is not adequate, which means there may be cell damage or overload. Therefore, kidney damage by toxicity can be detected earlier using measurements of proteins in urine.

Low molecular weight proteins that represent tubular damage are β2-microglobuluin, α1-microglobulin, retinol-binding protein which transports retinol from liver to other organs and cystatin-C, an inhibitor of cysteine proteinase.

c. Kidney Injury Molecule-1 (KIM-1)

KIM-1 is a type I transmembrane glycoprotein. KIM-1 is found in S1, S2 and S3 segments of PCT in case of ischemic and toxic AKI in human. Basal expression of KIM-1 is undetectable or very negligible amount in healthy kidney, but it significantly gets elevated in a dedifferentiated and regenerating proximal convoluting tubule. *When the kidney is exposed to toxic substances such as cisplatin, gentamicin or gets damaged by ischemia or reperfusion, KIM-1 can be used as a more sensitive biomarker than traditional nephrotoxic biomarkers such as BUN, serum creatinine, and proteinuria.* KIM-1 expression has been reported to correlate with proximal tubular injury, renal tubular regeneration and immune response by nephrotoxicants. *Both the FDA and EMA consider KIM-1 to be a highly sensitive biomarker for detecting acute drug-induced injury during drug development.*

d. Cystatin C

The protease inhibitor Cystatin C is a non-glycosylated low molecular weight protein. *Cystatin C has been proposed to be a marker as it is produced by all nucleated cells at a constant rate and is freely filtrated by the glomeruli and completely catabolized in the proximal tubules.* In a healthy state, a negligible amount of Cystatin C is found in urine. *Urinary CysC is a validated and qualified biomarker of acute tubular and glomerular injury in rats and it increases during impaired reabsorptive capacity of PCT.* Blood levels of Cystatin C is unaltered with muscle mass, exercise, sex, age of the individual etc. Hence, it is considered as a more realistic marker for glomerular function assessment even in cachexia or early acute kidney injury(AKI), where serum creatinine could underestimate the true renal function. *CysC is considered as a specific marker for GFR assessment than primary marker for AKI although it can be used to detect AKI.*

e. Clusterin

Clusterin is a disulfide-linked heterodimeric protein of 75 k Da, consisting of α- and β-subunits. It is present in many physiologic fluids and over expressed in kidney and urine of various species of animals in pre-clinical AKI, tissue remodeling, unilateral ureter obstruction, or subtotal nephrectomy models of kidney injury. Like KIM-1, Clusterin is expressed in damaged tubular cells

respond to stress and seen in a higher level in polycystic kidney disease and renal carcinoma. *Expression of clusterin is noticed in necrotic tubules with high-dose of paraminophenol administration in rats, often with higher intensity at the apical pole of the epithelium. Clusterin gene over expression was induced by different types of kidney injury in glomeruli, tubules and papillae of rats and dogs as a result of drug nephrotoxicity, surgery and ischemia, and renal diseases.*

f. NeutrophilGelatinase-Associated Lipocalin (NGAL)

NGAL is a 25 KDa glycoprotein which is covalently bound to neutrophil-gelatinase of the lipocalin family, and it contains eight beta strands forming a β-barrel in a closed cup. It is minimally expressed in lungs, stomach, colon and epithelial cells located in the PCT and neutrophils. *NGAL will increase if the thick ascending limb of the loop of Henle, distal tubule and collecting duct is damaged in rodents and humans.* NGAL level in urine and serum is correlated to serum creatinine level and the increase precedes increased serum creatinine level. EMA and US FDA encouraged the conduct of nonclinical and exploratory clinical analyses to evaluate the translational relevance of changes in urinary NGAL values and recommend evaluating urinary NGAL in early clinical studies and prospectively discuss proposed application of the clinical biomarker to decisions during the course of the study for clinical trial authorization.

8. Risk Assessment

Most risk assessment decisions are currently based on information concerning the aminoglycosides, halogenated anaesthetics, several heavy metals, and lithium, where there is an excellent concordance between animal data and findings in humans exposed to these agents. This has provided some predictive indication of what will take place in humans exposed to analogues of these compounds. On the other hand, the demonstration that the occurrence of light hydrocarbon-related adenocarcinomas is specific to male rats shows that there are examples where the molecular understanding of a renal lesion in animals is irrelevant to humans.

There are also therapeutic agents where attempts to extrapolate from animals to man have not been as successful. These include compounds such as cyclosporin, analgesics and non-steroidal anti-inflammatory agents. There are, however, a number of chemicals, such as renal carcinogens, mycotoxins, other natural toxins, and anti-cancer drugs, and some types of lesion, such as the immunenephropathies, where it has been difficult to establish good models in animals. *A host of chemicals alter glomerular filtration rate (GFR) or some other aspect of renal function, but the long-term health significance is still not known, and it is uncertain how to extrapolate such data to man.*

The impact of environmental chemicals on public health and clinical well-being has long been recognized, with a historical focus on heavy metals and molecules that are produced in the workplace. Increasing data have, however, indicated that the general public is unknowingly exposed to a wide range of chemicals as a consequence of normal consumer activities. These activities include dietary intake of food, domestic and commercial food preparation, household maintenance procedures, and routine medical and dental care.

A major food safety incident in 2009 exemplified the potential scope of the adverse renal consequences that can occur following population-wide exposure to organic contaminants. *Melamine is an organic nitrogenous compound used in the industrial production of plastics, dyes, fertilizers and fabrics that was considered safe on the basis of standard animal studies. This molecule was deliberately added to diluted raw milk at milk-collecting stations in China, with the presumed aim of falsely elevating assay results for protein content.*Melamine was later detected in numerous food and milk-containing products that were exported from China to many countries worldwide. *More than 300,000 infants and children exposed to milk-based formulas contaminated with melamine developed radiolucent stones, as well as impaired renal function and renal growth. T*he long-term consequences of melamine exposure in infancy and early childhood remain unknown, but exposure to melamine during adulthood might increase the risk of urolithiasis. The melamine story, therefore, provides a striking cautionary note regarding the potentially serious adverse consequences of exposure to environmental organic chemicals during normal consumer activity.

9. Conclusions

Human beings are exposed to various potentially toxic agents and conditions in their natural and occupational environments. The kidney, due to its concentrating ability and excretory function, is highly vulnerable to the effects of environmental toxins. *The high vulnerability of the kidney can be explained by its main physiological features, such as the highest blood flow per 100 g tissue, the largest endothelial surface by weight, highly active multiple metabolizing enzyme systems, the high concentration of filtered chemicals in tubular fluid adjacent to tubular cells, protein unbinding of chemical compounds in the tubules and further intrarenal biotransformation of chemicals.* Two major mechanisms inducing renal toxicity are (i) proximal tubular cell damage occurring due to extensive cellular uptake of toxins by both apical and basolateral transport systems and (ii) extensive tubular crystal formation due to the kidney's concentrating capacity.

The kidney plays a prominent role in mediating the toxicity of numerous drugs, environmental pollutants, and natural substances. Drugs known to be nephrotoxic include several cancer therapeutics, drugs of abuse, antibiotics, and radio contrast agents. Environmental pollutants known to target the kidney include cadmium, mercury, arsenic, lead, trichloroethylene, bromate, brominated-flame retardants, diglycolic acid, and ethylene glycol. Natural nephrotoxicants include aristolochic acids and mycotoxins such as ochratoxin, fumonisin B1, and citrinin.

Various preclinical studies suggested the use of Kim-1, NGAL, Clusterin, Cystatin, microalbumin, osteopontin, and N-acetyl glucosamine as specific urinary biomarkers in evaluating nephrotoxicity as compared to traditional markers like serum creatinine and blood urea nitrogen which are non-specific and insensitive. Use of these biomarkers in preclinical toxicity studies enables detection of potential nephrotoxicity issues or adversity assessment related to kidney toxicity during the screening of candidates as well as monitoring kidney functions in Clinical trials. *FDA and EMEA recommended using Kim-1, NGAL,*

and Osteopontin as validated biomarkers in clinical studies to assess the risk of nephrotoxicity.

Questions

1. *Define Nephrotoxicity?*
2. *Discusss about why the kidney is a target both for chemicals that are pharmacologically active and for toxic material?*
3. *What are the functions of a Nephron?*
4. *There are well over _____ morphologically different cell types in the kidney*
5. *The driving force for filtration is provided by _____, minus both the plasma osmotic pressure and the hydrostatic pressure in the Bowman's space*
6. *The proximal tubule plays a decisive role in maintaining _____*
7. *The medulla can be divided into the outer medulla and the inner medulla, the free part of which is referred to as the"_____".*
8. *Collecting ducts consists of three identifiable segments, which lie, respectively, in the _____, the outer medulla, and the inner medulla.*
9. *The distal tubule connects the thick ascending limb of the loop of Henle to that part of the collecting duct which originates in the _____.*
10. *The xenobiotic-transformation capacity of the kidney is about _____-_____% (depending on the system, species, and source of data) of that found in the liver.*
11. *Oxidases can convert chemicals into active intermediates or generate reactive species by _____.*
12. *The S2 proximal segment has a _____ concentration that is 2-3 times higher than the S1 or S3 segments.*
13. *Human kidneys have _____ and high GT2 activities, while rabbit kidneys have all three isoenzymes*
14. *The two kidneys process _____% of the resting cardiac output via an arterial blood supply.*
15. *Many organic acids and bases (including many drugs) are secreted (and reabsorbed) by _____processes located principally in the proximal tubule*
16. *Name the biomarkers for assessment of nephrotoxicity?*

--------End of the Document----------

About ToxGurukul

ToxGurukul is a group of professionals in the field of toxicology who are in search of a platform to learn and share the vast knowledge in this area. This syndicate belongs to independent professionals from different backgrounds of toxicology who share their knowledge to un-puzzle the Rubik's cube that each face in their daily work routine.

Website: www.toxgurukul.org.in

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