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Contemporary Review

CONTEMPORARY REVIEW

Nephrotoxicity and Renal Pathophysiology: A Contemporary Perspective

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ABSTRACT

The kidney consists of numerous cell types organized into the nephron, which is the basic functional unit of the kidney. Any stimuli that induce loss of these cells can induce kidney damage and renal failure. The cause of renal failure can be intrinsic or extrinsic. Extrinsic causes include cardiovascular disease, obesity, diabetes, sepsis, and lung and liver failure. Intrinsic causes include glomerular nephritis, polycystic kidney disease, renal fibrosis, tubular cell death, and stones. 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 radiocontrast 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. There are several common characteristics between mechanisms of renal failure induced by nephrotoxicants and extrinsic causes. This common ground exists primarily due to similarities in the molecular mechanisms mediating renal cell death. This review summarizes the current state of the field of nephrotoxicity. It emphasizes integrating our understanding of nephrotoxicity with pathological-induced renal failure. Such approaches are needed to address major questions in the field, which include the diagnosis, prognosis and treatment of both acute and chronic renal failure, and the progression of acute kidney injury to chronic kidney disease.

Key words: kidney; nephrotoxicity; renal pathology; acute kidney injury; chronic kidney disease; renal failure.

Nephrotoxicity can be defined as the adverse effect of substances on renal function (Perazella, 2009). These substances 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 (Zhou *et al.*, 2008).

A somewhat complicated staging system exists for the assessment of renal injury (see below). Time is a key consideration between acute kidney injury (AKI) and chronic kidney disease (CKD), both in terms of the rate of functional decline and the length of time that renal function is decreased. The terms AKI and CKD represent a relatively newer way to refer to the historical terms of acute renal failure (ARF) and chronic renal failure (CRF).

Renal pathology focuses on the diagnosis and characterization of medical diseases of the kidney that are non-tumor related. The damage to the kidney does not have to be induced by chemicals, thereby distinguishing renal pathology from nephrotoxicity. Further, kidney damage induced by pathological events

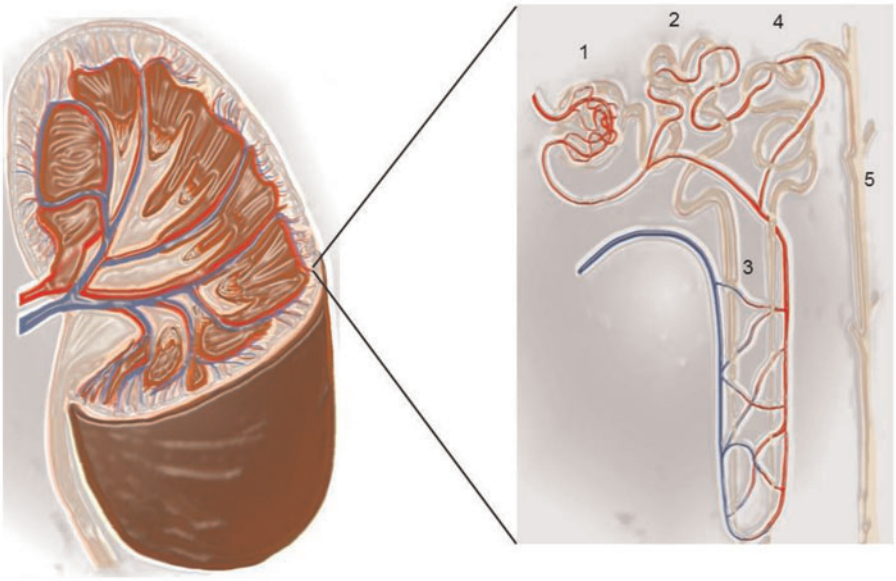

Figure 1. Characterization of the structure of the kidney (A). The inset demonstrates the structure of the nephron (B), the functional unit of the kidney. The numbers in B refer to individual structures of the nephron, including the glomerulus (1), the proximal tubules (2), the Loop of Henlee and its vasculature (3), the distal tubules (4), and the collecting ducts (5).

can be induced by extrinsic events such as hypertension, obesity, sepsis, liver failure, and diabetes.

Despite the differences in definitions, there are several commonalities between nephrotoxicity and renal pathology. The foremost of these is that both are primarily caused by renal cell death and involve changes in the structure of the functional unit of the kidney called the nephron (Figure 1). This includes changes to the tubules, glomeruli, the interstitium and the intra-renal blood vessels (Makris and Spanou, 2016). 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.

The mechanisms mediating renal cell death induced by nephrotoxicants and renal pathologies are strikingly similar. For example, ischemia-induced AKI involves ATP depletion, oxidative stress, proximal tubule cell death and loss of the brush border membrane, and cell polarity (Devarajan, 2006). In comparison, AKI induced by the cancer chemotherapeutic and prominent nephrotoxicant cisplatin also involves oxidative stress, proximal tubule cell death and loss of the brush border membrane and polarity (Ludwig *et al.*, 2004; Miller *et al.*, 2010). Increased oxidative stress, loss of ATP and proximal tubule cell death are also commonly seen in nephrotoxicity induced by contrast media, which is also known to alter glomerular function and renal blood flow (Calvin *et al.*, 2010). This is similar to events that are seen in diabetic-induced nephropathy (Hakim and Pflueger, 2010). Proteinuria, glomerular fibrosis and interstitial fibrosis are key events in renal failure induced by hypertension and hyperglycemia, which shares some common features with glomerular alterations induced by D-pencillamine, furosemide, and gold.

The characterization of kidney damage and its staging are also the same regardless of whether the injury is caused by a nephrotoxicant or a renal pathology. Although a complete review of renal pathology is impractical at this time, there are several definitions and terms that anyone studying nephrotoxicity should be familiar with. The reader is referred to recent reviews that have covered this topic in detail (Linkermann *et al.*, 2014; Makris and Spanou, 2016).

Kidney Damage, AKI, and CKD

Kidney damage can be defined as changes in the function or structure of the kidney, even in the absence of initial changes in the GFR. An important addendum to this definition is that these changes can lead to decreases in GFR over time. The most prominent marker of kidney damage is proteinuria (Makris and Spanou, 2016). Kidney damage may also progress to AKI or CKD. In some cases kidney damage may be repaired, but the mechanisms mediating this repair are not as well understood as the mechanisms of renal cell injury (El Sabbahy and Vaidya, 2011).

The approach to defining kidney injury has changed over recent years, and multiple definition schemes exist. These include the Kidney Disease Global Outcomes (KDIGO) definition and staging study and the Risk, Injury, Failure, Loss and End-stage renal disease (RIFLE) designation, along with its modifications (Makris and Spanou, 2016). A key difference between AKI and CKD in both of these criteria is the length and rate of time in which renal function is declined, with CKD being defined as lasting longer than 3 months based on structural and functional abnormalities (McManus and Wynter-Minott, 2017).

In contrast to CKD, AKI is an abrupt change in renal function and is commonly defined by changes in BUN (azotemia) and/or sCr. Proteinuria will also be seen. AKI can last hours to weeks. AKI may also resolve, resulting in the return of clinical markers to basal levels, or AKI can worsen leading to renal dysfunction and possibly multi-organ dysfunction (Hultstrom *et al.*, 2018). The assessment of AKI is primarily based on changes in GFR, which is initially based on changes in sCr levels and urine output, although some studies use BUN as an indicator (Fuchs and Hewitt, 2011). An intense level of research has been put into identifying new biomarkers of AKI; however, given that AKI is

rarely induced by a single etiology, it is not known if a single biomarker would be able to reflect AKI induced by all stimuli. This is true even for GFR as kidney damage can occur in the absence of decreases in GFR (Hultstrom *et al.*, 2018). This fact reflects a challenge in the field.

Like AKI, CKD has several classifications that differ based on the presence or absence of kidney damage, urine output, hypertension and changes in GFR (McManus and Wynter-Minott, 2017). A standard reference point for GFR in staging CKD is a value equal to or below 60 ml/min/1.73 m^2 for >3 months. It should be noted that a decrease in GFR below this level only suggests CKD, and does not indicate if the kidney injury is the result of nephrotoxicants, renal pathology or extra-renal events.

CURRENT FOCUSES OF NEPHROTOXICITY

Biomarkers

The fact that kidney damage and structural changes can occur without any major changes in GFR, and that GFR varies with age, gender and race, has resulted in a significant amount of research into novel biomarkers of renal damage. The overall goal of this research is to identify biomarkers that are more sensitive than the established markers and that are more indicative of pre-renal damage (ie, before decreases in GFR). Research is also focused on identifying biomarkers that can indicate the nature of the mechanisms involved. Efforts are also being made, with some success, to identify biomarkers that identify the specific site along the nephron that is injured (Fuchs and Hewitt, 2011) (Table 1). More recent studies have focused on microRNA in the urine, and assessment of exosomes and other extracellular vesicles (Cardenas-Gonzalez *et al.*, 2017; Erdbrugger and Le, 2016; Ichii and Horino, 2018; Street *et al.*, 2017). Table 1 lists classical and emerging indicators of nephrotoxicity, as well as several classical nephrotoxicants.

Mechanisms

Numerous studies have focused on the biochemical and molecular mechanisms of nephrotoxicity. These studies, coupled with bioinformatic-based approaches mean that we know more than ever about the mechanisms by which nephrotoxicants induce renal cell death. These studies have aided the rise of the concept of in-common mechanisms, which are similar to damage associated molecular patterns, or DAMPs (Hultstrom *et al.*, 2018). The basic idea is that similar mechanisms of action will be induced by nephrotoxicants, even if these nephrotoxciants are structurally diverse. Eventually the mechanisms activate pathways characteristic to the cell undergoing injury. Although DAMPs are not specific to the kidney, the combination of DAMPs with classical and emerging biomarkers of renal function will be important to addressing key challenges in the field (see below).

Significant effort has gone into studying the mechanisms of nephrotoxicant-induced cell death. All 3 major types of cell death occur in renal cells including apoptosis, autophagy and necrosis. The mechanisms of apoptosis include both intrinsic and extrinsic pathways and several cancer therapeutics, antibiotics, fungi and mold, metals such as mercury, and oxidants are known to induce renal cell apoptosis (Mkaddem *et al.*, 2010; Rana, 2008; Servais *et al.*, 2008). Autophagy has not received as much attention as apoptosis in the kidney. Those studies that do exist show that several cancer therapeutics, antibiotics, fungal agents, and molds induce autophagy (Decuypere *et al.*, 2015; Kimura *et al.*, 2017; Kitada *et al.*, 2017; Tang *et al.*, 2018). Many of these same studies also suggest that autophagy may be renal-protective. Further studies are needed to answer this question and to identify the key signaling events that determine if autophagy is either nephrotoxic or nephro-protective. Autophagy in the kidney is an exciting area of research, as its signaling pathways may be unique sites for therapeutic intervention in AKI (Kaushal and Shah, 2016).

Several compounds are known to induce necrosis in the kidney. Many of these compounds can also induce apoptosis or autophagy. The exact mechanism is dependent on the cell type involved, the dose and the length of exposure, which is true of all toxicants. The field of necrosis has undergone a renaissance of late, leading to the study of programed or regulated necrosis (Linkermann *et al.*, 2014; Sancho-Martinez *et al.*, 2015). Programed necrosis involves several pathways, including those that involve the activation of select caspases, an event historically associated with apoptosis. Other pathways activated during programed necrosis include those involving receptor- and kinase mediated pathways, such as the receptor interacting protein kinase and mixed lineage kinase pathways (Martin-Sanchez *et al.*, 2018). This also includes the formation of the inflammasome. Programed necrosis has been heavily studied in other systems, but has received relatively less attention in the kidney. As such, significant opportunities exist for exploration.

Many studies of nephrotoxicants focus on a single chemical or a single cell. Although such studies are necessary to understand the mechanism of action, they may not be truly reflective of the *in vivo* situation where the effect of damage at one site of the nephron is propagated to other sites. Further, the complexity of AKI and CKD are such that rarely do patients get exposed to a nephrotoxicant without some mitigating factor, such as hypertension, obesity, liver, lung or heart problems, or the presence of other chemical, such as alcohol or cigarette smoke. Thus, more studies are needed that assess the effects of mixtures and comorbidities. Further, additional studies are needed assessing how the mechanisms of nephrotoxicant-induced cell death depend on gender and age. This last point is extremely relevant, as a high number of patients that develop AKI or CKD are children or the elderly.

Oxidant-Induced Renal Injury and the Role of Mitochondria

The kidneys are susceptible to oxidant-induced injury due their high reliance on mitochondria and ATP to facilitate the transport function of the nephron. This is especially true of the proximal tubule cells. As such, the mechanisms of oxidant-induced injury have been a focus of nephrotoxicity for decades. Although a significant amount of work focused on changes in mitochondrial function and oxidative stress responsive proteins, recent focus has shifted to oxidant-induced cell signaling events, such as the Nuclear factor (erythroid-derived 2)-like 2 (NRF2)/Kelch-like ECH-associated protein 1 (KEAP) pathways (Liu *et al.*, 2009; Nounou *et al.*, 2016). Such studies are impactful because oxidative stress is a key event in extra-renal pathologies as well.

The role of the mitochondria in mediating renal cell death has also been heavily studied, but continues to produce new and exciting findings (Bhargava and Schnellmann, 2017). This is especially true in the proximal tubules, which have a high abundance of mitochondria to facilitate their transport and secretory functions. The mitochondria are also a key site for mediation of cell death via the release of proapoptotic inducers and the production of ATP, which is a master regulator in the decision of a cell to die by apoptosis, necrosis, or autophagy (Linkermann *et al.*, 2014). Recent studies have focused on identifying accurate determinates of mitochondrial function as markers for renal cell death. Studies have also focused on approaches to either

Table 1. Classical and Emerging Indicators of Nephrotoxicity

Biomarker	Species	Nephrotoxicant	Nephron Segment	References
Albumin	Rat Human Monkey	Cisplatin Gentamicin Puromycin Aminonucleosides	Glomerulus Proximal tubule	Shin *et al.* (2014), Uchino *et al.* (2017)
α-GST	Rat Human	Cisplatin Polymixin	Proximal tubule	Keirstead *et al.* (2014), Shin *et al.* (2014)
α2-microglobulin	Rat Human Monkey	Gentamicin	Proximal tubule	Gautier *et al.* (2016)
Calbindin D28	Rat Human	Aristolochic acid Cisplatin	Distal tubule Collecting Duct	Fuchs *et al.* (2014), George *et al.* (2018)
Clusterin	Rat Human Canine Monkey	Gentamicin Bromate	Proximal tubule	Harpur *et al.* (2011), Kolisetty *et al.* (2013a,b), Zhou *et al.* (2014)
Cystatin-C	Rat Human Canine	Cadmium GPSH Gentamicin Aminoglycosides	Proximal tubule Distal tubule	Lau *et al.* (2017), Mohamed Ali *et al.* (2017), Mohamed *et al.* (2016), Sasaki *et al.* (2014)
Exosomal fetuin-A	Rat Human	Cisplatin	Proximal tubule	
Fibronectin	Rat	Cyclosporine	Proximal tubule	Carlos *et al.* (2014)
Glutamyl aminopeptidase	Rat	Cisplatin	Proximal tubule	Quesada *et al.* (2017)
HSP72	Rat	Cisplatin Acetaminophen	Proximal tubule Distal tubule	Perez-Villalva *et al.* (2017)
3-Indoxysulfate	Rat	Cisplatin	Proximal tubule Distal tubule	Won *et al.* (2016)
Interleukin-18	Rat Human	Cadmium	Proximal tubule Distal tubule	Mohamed Ali *et al.* (2017)
KIM-1	Rat Human Canine Monkey	Gentamicin Tacrolimus Cisplatin Glycophosphate surfactant-based herbicides Polymixin B Paraquat Acetaminophen Cyclosporine	Proximal tubule	Burt *et al.* (2014), Carlos *et al.* (2014), Cosner *et al.* (2015), Keirstead *et al.* (2014), Luo *et al.* (2016), Nan-Ya *et al.* (2015), Pavkovic *et al.* (2016), Pavkovic and Vaidya 2016), Shin *et al.* (2014), Wunnapuk *et al.* (2013, 2014)
Liver-type fatty acid-binding protein	Rat Human	Cisplatin	Proximal tubule	Andreucci *et al.* (2017), Kamijo-Ikemori *et al.* (2013)
Monocyte chemotactic protein-1	Human	Cisplatin	Proximal tubule	George *et al.* (2018)
NAG	Rat Human Canine Monkey	Gentamicin	Proximal tubule	Gautier *et al.* (2016), Zhou *et al.* (2014)
Netrin-1	Rat Human	Cisplatin Gentamicin Lipopolysaccharide	Proximal tubule	Reeves *et al.* (2008)
NGAL	Rat Mouse Human Monkey Canine	Cisplatin Gentamicin Polymixin B Aristolochic Acid	Proximal tubule Distal tubule	Burt *et al.* (2014), Cosner *et al.* (2015), Fuchs *et al.* (2014), Luo *et al.* (2016), Phillips *et al.* (2016), Seker *et al.* (2015), Uchino *et al.* (2017), Zhou *et al.* (2014)
OAT5	Rat	Cisplatin	Proximal tubule	Bulacio *et al.* (2015)
Osteopontin	Rat Human Monkey	Aristolochic Acid Cyclosporine A Gentamicin	Proximal tubule Loop of Henlee Distal tubule	Carlos *et al.* (2014), Fuchs *et al.* (2014), Gautier *et al.* (2016), Phillips *et al.* (2016)
Pyruvate Kinase M2	Rat	Cisplatin	Proximal tubule	Cheon *et al.* (2016)

Table 1. (continued)

Biomarker	Species	Nephrotoxicant	Nephron Segment	References
Renal papillary antigen-1	Rat	BEA Propyleneimine Indomethacin	Collecting duct	Fuchs *et al.* (2014)
Selenium-binding protein 1	Rat	Cisplatin Mercury	Proximal tubule	Kim *et al.* (2017), Lee *et al.* (2017)
Trefoil factor 3	Human	Cisplatin	Proximal tubule Distal tubule	Fuchs *et al.* (2014), George *et al.* (2018)
Tumor necrosis factor-α	Rat	Cyclosporine	Proximal tubule	Carlos *et al.* (2014)
TMIP-1	Rat Human	Cisplatin	Proximal tubule	Fuchs *et al.* (2014), Shin *et al.* (2014)
TIMP-2	Human	Cisplatin	Proximal tubule	Schanz *et al.* (2017)
TIMP2-IGFBP-7	Human	Cisplatin	Proximal tubule	Schanz *et al.* (2017)
Vanin-1	Human	Cisplatin	Proximal tubule	Hosohata *et al.* (2016)
Vascular endothelial growth factor	Rat Human	Cisplatin	Proximal tubule Distal tubule	Fuchs *et al.* (2014), Shin *et al.* (2014)

preserve mitochondrial function or enhance biogenesis to protect against nephrotoxicity (Bhargava and Schnellmann, 2017; Chen *et al.*, 2017; Collier *et al.*, 2016; Geng *et al.*, 2017; Ni *et al.*, 2017; Zhang *et al.*, 2017). Notable nephrotoxicants whose mechanisms of action are reported to involve mitochondrial dysfunction include cisplatin, ethylene glycol, diglycolic acid, and metabolites of trichloroethylene, such as S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) (Hovda *et al.*, 2010; Landry *et al.*, 2011, 2013; Lash *et al.*, 2003; Lock and Schnellmann, 1990; Peres and da Cunha, 2013).

Transporters

Transporters have always been a primary focus of nephrotoxicity research (George *et al.*, 2017). Transporters are present in both the apical and basolateral membrane of cells along the nephron. They can either transport substances out of the kidney back into the blood (reabsorption), or they transport substances into the cells and eventually into the filtrate (secretion). The expression of transporters varies along the nephron and is species-, and possibly gender-dependent (Breljak *et al.*, 2016). Transporters are known to influence the mechanisms of toxicity induced by several nephrotoxicants, including cancer therapeutics, antibiotics, fungal agents, and environmental pollutants (George *et al.*, 2017). Transporters in the kidney are also believed to be very relevant to the nephrotoxicity of metals such as mercury (Aleo *et al.*, 2005; Lash *et al.*, 2005; Zalups and Ahmad, 2004, 2005).

The variation in expression of transporters between species and cell lines results in some uncertainty in assessing the risk of nephrotoxicity to humans. Alterations in transporter function in several renal pathologies are also reported, and select transporters are known to be pharmacological targets for the treatment of several diseases including diabetes, hypertension and diabetes insipidus (Bachmakov *et al.*, 2008; Brosius and Heilig 2005; Rojek *et al.*, 2005; Thomas *et al.*, 2003).

Several studies have shown that single nucleotide polymorphisms (SNPs) exist for several renal transporters, such as the multidrug resistance-associated protein 2 (MRP2), the multidrug and toxin extrusion protein 1, the organic cation transporters 1 and 2, members of the organic anion transporter (OATs) family, and P-glycoprotein (Pgp) (Chang *et al.*, 2017a; Filipski *et al.*, 2009; George *et al.*, 2017; Hawwa *et al.*, 2009; Meyer *et al.*, 2017; Naesens *et al.*, 2009). These SNPs may account for idiosyncratic responses of individuals to nephrotoxicants as well as variability in patient response to therapeutics that target these transporters. In support of this hypothesis, there are studies reporting correlations between the presence of SNPs in several of these transporters and increases in biomarkers of AKI such as kidney injury molecule-1 (KIM-1) in response to cisplatin (Chang *et al.*, 2017a). Further the variability in nephrotoxicity induced by drugs such as cyclosporine and tacrolimus have been correlated to SNPs in Pgp (Naesens *et al.*, 2009). Genetic polymorphisms in MRP4 have been shown to alter the transport of methylated arsenic metabolites *in vitro* (Banerjee *et al.*, 2016), suggesting that genetic polymorphisms in renal transporters can also mediate the nephrotoxicity of environmental pollutants.

Glomerulonephritis

Glomerulonephritis is inflammation of the glomerulus and can be induced by both nephrotoxicants and renal pathologies. Nephrotic disease, which is indicated by higher levels of proteinuria, can be immune-based and alters the architecture and function of epithelial cells of the podocytes of the glomerulus. In contrast, nephritic syndrome is more inflammatory-based, with higher levels of cellular proliferation and changes in the glomerular basement membrane and hematuria. Both can involve immune complex deposition, although IgA nephropathy is more common with nephritic syndrome.

Nephrotoxicants can induce glomerular diseases, as can several renal pathologies, including diabetes, obesity, hypertension and systemic lupus erythematosus (Nozaki *et al.*, 2012; Selvaskandan *et al.*, 2018; Turner *et al.*, 2006). Although the role of immune complexes and inflammation are well described, therapeutic treatments are still somewhat lacking (Dickinson, 2016). The recent explosion in immune-based therapeutics and biologics raises opportunities for similar approaches in the kidney, but also raises concerns about nephrotoxicity as well.

Bioactivation

The role of drug metabolism in the mechanism of action of nephrotoxicants has been intensely studied. In fact, many nephrotoxicants are already metabolites themselves routed to the kidney from organs such as the liver (Cummings and Lash, 2000; Cummings *et al.*, 2000c). This includes metabolites of arsenic (Banerjee *et al.*, 2016). There are also many nephrotoxicants whose mechanisms of action are dependent on kidney-specific

metabolism. A full review of renal xenobiotic-metabolizing enzymes is not practical here, but the reader is referred to several reviews (Lock, 1994; Müller *et al.*, 1999; Pavek and Dvorak, 2008). Importantly, phases I and II enzymes are expressed along the nephron and the expression of these enzymes is species-dependent. These enzymes include cytochrome-P450 monooxygenases, flavin-containing monooxygenases (FMO's) and glutathione S-transferases (GST's). Differences in the expression of these enzymes along the nephron may account for the cell-dependent differences in toxicity of several nephrotoxicants as well as species-dependent differences. Examples of nephrotoxicants known to have their mechanisms of action depend on the activity of cytochrome-P450 monooxygenases include chloroform and acetaminophen (Fahrig *et al.*, 1995; Hart *et al.*, 1994, 1995; Hoivik *et al.*, 1995; Hu *et al.*, 1993; Lash, 1994; Smith, 1986; Smith *et al.*, 1984). Examples of nephrotoxicants that are metabolized by FMOs) include DCVC (Lohr *et al.*, 1998; Mani and Kupfer, 1991; Ripp *et al.*, 1997, 1999). Examples of nephrotoxicants metabolized by GST's include $HgCl_2$ (Zalups, 1995), halogenated alkenes and aromatics, and possibly acetaminophen (Birge *et al.*, 1990; Koob and Dekant, 1991; Lash, 1994; Monks *et al.*, 1994). The toxicity of the halogenated alkene trichloroethylene to rat and human kidneys is believed to be a result of conjugation with GSH to form S-(1, 2-dichlorovinyl)glutathione, and the subsequent processing to DCVC in renal proximal tubular cells (Cummings and Lash, 2000; Cummings *et al.*, 2000b).

Models

Rodents continue to be the mainstay for *in vivo* studies of nephrotoxicity. However, rats and mice have significant drawbacks when it comes to assessing nephrotoxicity. One of these is the expression of α2u microglobulin. The expression of α2u microglobulin is also species- and gender-dependent (Lehman-McKeeman, 1993; Lehman-McKeeman and Caudill, 1994). It is significantly higher in rats then mice, but relatively lower, if not nonexistent, in humans. Further, α2 u microglobulin expression is gender-dependent in that it is specific to male rats (Lehman-McKeeman, 1993). These differences are believed to account for the higher toxicity of several nephrotoxicants in rats as compared with humans, especially after long-term exposures. These differences are also believed to account for differences in susceptibility between male and female rats and serve as a classical example of the need to study gender differences in the susceptibility to nephrotoxicants.

In vitro systems for studying nephrotoxicity are varied. They range from immortalized cell lines from rats, mice, dogs, pigs, and humans to primary cultures of renal cells and kidney slices. Some of the more commonly used human cell lines include immortalized human proximal tubular cells, or HK-2 cells (Iwata *et al.*, 1994; Sohn *et al.*, 2013). HK-2 cells are representative of cortical cells, but, like most cell lines, are immortalized and are likely not fully representative of the *in vivo* state. Normal rat kidney cells and their variations are commonly studied and do appear to somewhat reflect the *in vivo* situation for some nephrotoxicants in rats, at least with regards to susceptibility. Other cell lines commonly used include primary human proximal tubular epithelial cells (Adler *et al.*, 2015; Huang *et al.*, 2015), engineered kidney cells from reprogramed fibroblast or induced pluripotent stem cells (Kaminski *et al.*, 2017), porcine kidney (LLC-PK1), and canine kidney (MDCK) cells (Hostetler, 1984; Hostetler and Richman, 1982; Mertens *et al.*, 1990; Troyer *et al.*, 1986). The advantage of these cells is the ability to conduct in-depth mechanistic studies using molecular approaches, and that they allow for high-throughput studies (Tiong *et al.*, 2014).

Freshly isolated cells and primary cultures of rats, mice, rabbits and human renal cells have been utilized for the study of nephrotoxicants for decades. These cells have the advantage of being more closely aligned with the *in vivo* state and have energetics that are more representative of cells along the nephron depending on the media and buffer used (Adler *et al.*, 2015; Gozalpour and Fenner, 2018; Rodeheaver *et al.*, 1990; Tiong *et al.*, 2014). These cells have the disadvantage that long-term or chronic studies can be somewhat difficult. Further, while primary cultures can be used over multiple passages, they do not always retain protein and gene expression representative of the *in vivo* state (Cummings *et al.*, 2000a). Primary cultures can also be somewhat laborious and expensive to isolate and maintain.

A middle ground for studying nephrotoxicity is the use of renal slices. This approach was heavily used for the initial studies of many classical nephrotoxicants and is still used today (Genovese *et al.*, 2016; Stribos *et al.*, 2016, 2017). One advantage of these models is that the nephron structure is maintained, as is the expression of transporter and drug metabolism proteins. A disadvantage is that longer term (ie, chronic) studies are somewhat impracticable.

There is still no perfect *in vitro* or *ex vivo* model for studying nephrotoxicity in the human kidney. However, several advances have been proposed. Most recently, studies have proposed the use of kidney on a chip and microdialysis (Gozalpour and Fenner, 2018; Lee and Kim, 2018). These models have the advantage of allowing for studies under dynamic flow environments. Further, in some cases cells can be seeded that represent the sequence found along the nephron (Figure 1), allowing for recapitulation of the exposure *in vivo*. There are also reports of chip models combining liver and kidney cells, allowing for the assessment of the role of hepatic-bioactivation on nephrotoxicity (Chang *et al.*, 2017b). Other studies have suggested the use of renal spheroids allowing for assessment of the role of the microenvironment on nephrotoxicity (Bao *et al.*, 2018; Grange *et al.*, 2017; Karpman *et al.*, 2017; Qu *et al.*, 2018). With regards to alternative models, studies have assessed the nephrotoxicity of several compounds using models such as xenopus oocytes, zebrafish, and *Caenorhabditis elegans* (Droz and McLaughlin, 2017; Ganner and Neumann-Haefelin, 2017; Morales and Wingert, 2017; Sirac *et al.*, 2018). Although such studies may not completely reflect the human kidney, they do have the advantage of being amicable to gene editing to humanize them, such as CRISPR-Cas9 and other approaches.

FUTURE DIRECTIONS

A significant amount of data on the mechanisms of AKI and nephrotoxicity has been produced. Unfortunately, much of the knowledge gleaned from these data has not fully translated to the clinic. Perhaps the greatest support for this opinion is the fact that there has been no improvement in the mortality of hospitalized patients with AKI over the last 40–50 years. For example, a retrospective study assessing the occurrence of AKI from the 1970s to 2002 reported that 50% of patients with AKI (called ARF in the study) died prior to discharge (Mehta *et al.*, 2002). A review summarizing several recent studies (Makris and Spanou, 2016) reported that the prevalence of AKI in "community" patients was around 4.3%. This value increased to approximately 15% in hospitalized patients, and to 60% in critically ill patients. This same review reported that mortality exceeded 60% in patients developing severe AKI (ie, those requiring renal replacement therapy). Thus, the front line has not budged in almost 50 years. This fact alone demonstrates that a

significant public health problem exists and that challenges still remain to be addressed. This is not to say that the last 50 years of research has not been fruitful. We know more about the mechanisms mediating AKI, especially toxicant-induced AKI, than ever before. Further, studies on both toxicant- and pathological-induced AKI have shown several common causes, including glomerular nephritis, oxidative stress, mitochondrial dysfunction, and hydronephrosis (Hultstrom *et al.*, 2018). Studies utilizing transcriptomics, proteomics, and lipidomics with classical nephrotoxicants have also demonstrated DAMPs and "in-common" mechanisms similar to both pathological- and toxicant-induced renal injury.

Although studies examining AKI induced by novel drug candidates or environmental pollutants will always be needed, emphasis is also needed on identifying molecular targets for therapeutic intervention of AKI. In this regard, there are plenty of questions left to be answered. Listed below are just a few of these. These are certainly not the only questions worth studying, and the field remains fertile ground for several other areas, including those in epigenetics, metals toxicity, nanotoxicity, and unfolded protein response, to name just a few. Rather, the below questions address the overarching theme of this review of integrating our knowledge of nephrotoxicity with our knowledge of renal pathology to significantly advance our ability to predict, diagnose, prevent, and treat renal injury.

Better Biomarkers of Renal Injury

The last 10–15 years have seen tremendous advances in biomarkers of renal injury, with the charge primarily being led by the identification of KIM-1 (see Table 1). Nevertheless, these markers have yet to be heavily incorporated into the KDIGO, RIFLE, and Acute Kidney Injury Network (AKIN) designations. Additionally, several researchers are attempting to identify novel markers (see above). Studies are also focusing on biomarkers that identify specific sites of damage along the nephron, biomarkers whose levels are reflective of the time and duration of injury, as well as biomarkers that indicate the mechanisms and cause of renal failure (Bonventre *et al.*, 2010; Cardenas-Gonzalez *et al.*, 2017; Fuchs and Hewitt 2011; Gerlach and Vaidya, 2017; Selvaskandan *et al.*, 2018; Street *et al.*, 2017). Studies are also now focusing on comparing the sensitivity of newer biomarkers, such as KIM-1, neutrophil-gelatinase-associated lipocalin (NGAL), N-acetyl-β-glucosaminidase (NAG), clusterin and tissue inhibitor of metalloproteinase 1 (TIMP-1). In this regard, several studies suggest that KIM-1 is more sensitive than several other biomarkers (Espandiari *et al.*, 2007; Sieber *et al.*, 2009; Zhou *et al.*, 2008); however, this finding may be species-dependent. Although time combined with classical indicators of renal function remain the standard for assessing CKD, biomarkers that indicate the likelihood that AKI will progress to CKD would be incredibly useful.

Understanding How AKI Turns Into CKD

Although many studies have investigated the mechanisms by which nephrotoxicants induce CKD, there have been fewer investigating mechanisms mediating the transition from AKI to CKD. Several models exist for recapitulating CDK arising from pathologies (Bao *et al.*, 2018). These models have their advantages and disadvantages, but still may be useful for studying how AKI transitions to CKD. These models may also be useful for studying how patients with preexisting renal failure respond to nephrotoxicants.

In addition to models that recapitulate the transition of AKI to CKI, there is also a need for more relevant exposure protocols. Exposing mice to high levels of cisplatin for 3 days does not recapitulate chemotherapeutic-induced renal injury observed in cancer patients. The same is true for other toxicants. It can no longer be safely assumed that exposure of cells to high doses of toxicants for a short amount of time will yield the same mechanisms as exposure to lower concentrations for long periods of time.

Nephrotoxicity and Renal Pathology

It is somewhat unusual, although not rare, to see AKI or CKD induced in the absence of any other toxicant or comorbidity. Despite this, there are relatively fewer studies assessing the effect of nephrotoxicants in the presence of other chemicals, or in the context of other pathologies. This may be one reason why the occurrence of AKI in ICU patients, and the mortality rate of this AKI, has not changed in decades. Further, AKI and CKD often result in multiple organ dysfunction. Thus, more studies are needed assessing nephrotoxicant-induced AKI and CKD induced by mixtures or in the presence of other diseases, such as cancer, cardiovascular disease, obesity, or liver failure. As an example, the literature is filled with studies of the mechanisms of cisplatin-induced AKI; however, the majority of these studies are done in models that do not have cancer, and were initially in healthy adult animals. This doesn't mirror the clinical situation well as the majority of patients are in their later 50s and have tumors. The same can be said for patients developing AKI or CKD in conjunction with nonalcoholic steatosis, lung or heart failure, osteoporosis, or in patients that are heavy drinkers and smokers. These areas represent a logical next step to understanding the full impact of nephrotoxicants on the development of AKI and CKD.

CONCLUSIONS

The field of nephrotoxicity is alive and well. Despite a perceived decrease in the funding of studies focusing on toxicant-induced kidney injury, it should be noted that funding for pathological-induced kidney injury, such as that induced by diabetes or heart disease, remains strong. It is likely that this area will continue to grow as the obesity epidemic and opioid crisis worsen. As such, researchers in the field may want to shift their expertise from understanding the mechanisms of single agents alone, to understanding the mechanism of injury with comorbidities or in the presence of other chemicals and mixtures. Such studies should use clinically and environmentally relevant concentrations and adapt relevant exposure protocols. Further, these studies should also focus on the gender- and age-dependence of nephrotoxicity.

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