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Next-generation biomarkers for detecting kidney toxicity

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Abstract

There is a paucity of biomarkers that reliably detect nephrotoxicity. The Predictive Safety Testing Consortium (PSTC) faced several challenges in identifying novel safety biomarkers in the renal setting.

The kidney is a major site of organ damage caused by drug toxicity. This frequently manifests during drug development and/or in standard clinical care. Nephrotoxicity resulting from drug exposure has been estimated to contribute to 19–25% of all cases of acute kidney injury (AKI, the currently preferred term for the clinical disorder formerly called acute renal failure) in critically ill patients[1]. Given the societal cost of nephrotoxicity and the insensitivity of current methods to detect it, sensitive methods for prediction of toxicity in preclinical studies and identification of injury in humans are extremely important for patient safety in clinical practice and in all stages of the drug-development process. It is in the interest of patients, physicians, the drug industry and health regulatory bodies to prevent new nephrotoxic drugs from entering the market or, when the medical need dictates use of such an agent, to be able to identify early and best manage nephrotoxicity.

This article discusses the purview of the first effort of the PSTC—a collaboration of the biotech and pharmaceutical industry, the US Food and Drug Administration (FDA; Rockville, MD), the European Medicines Agency (EMEA; London, UK) and academia—to facilitate the qualification of renal biomarkers for safety in drug development. It brings together expertise from a variety of disciplines to organize and/or create evidentiary datasets to present to the regulatory agencies for qualification decision-making. Although this first

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published effort describes the rationale of the PSTC's Nephrotoxicity Working Group for identifying new renal safety biomarkers, the consortium also has working groups focused on hepatotoxicity, vascular injury and myotoxicity as well as genetic signatures for carcinogenicity. Much of what we discuss in the context of traditional small molecules also applies to nephrotoxicity arising from the use of alternative and complementary therapies, including herbs, natural products and nutritional supplements, especially when they are combined with conventional drugs[2].

The need for renal biomarkers

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. 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. However, it is important to recognize that safety concerns must always be incorporated into a general 'risk-benefit' analysis and that toxicity of a drug does not necessarily mean that it should not be developed or approved. Some examples of nephrotoxic drugs that have provided a very high therapeutic benefit are the aminoglycoside antibiotics, the cancer drug cisplatin and the antiviral tenofovir.

Some ideal attributes of markers of AKI are summarized in Box 1. The most useful biomarkers are those that can be used in animals and humans. These 'translational' biomarkers can be rigorously studied in animals, thereby establishing well-defined relationships between biomarker levels and kidney histopathology. One of the most notable challenges in assessing drug nephrotoxicity in humans is that we do not have tools capable of predicting nephrotoxicity across species boundaries.

Normally, when kidney injury is found in preclinical studies of one species and not in another, the compound being tested is not developed. The development of Bristol Myers Squibb's (Princeton, NJ) Sustiva (efavirenz) provides a good example of a situation in which the abandonment of a drug owing to species-specific differences in nephrotoxicity would have prevented many patients from benefiting from use of this non-nucleoside reverse transcriptase inhibitor for treating HIV infection. Sustiva causes renal epithelial cell necrosis in rats, but not in cynomolgus monkeys or humans[3]. Its toxicity in rats arises from a species-specific nephrotoxic glutathione-conjugated metabolite3. Unfortunately, however, when an explanation like this cannot be found, otherwise compelling drug candidates are routinely abandoned before introduction to humans.

Kidney injury associated with drug toxicity

The human kidney is a complex organ with approximately 1 million functional units called nephrons. The nephrons of two normal kidneys are collectively responsible for filtering approximately 150–180 liters of plasma per day and then processing the filtrate to regulate fluid, electrolyte and acid-base balance while eliminating waste products. The kidneys also produce hormones important for cardiovascular, hematologic and skeletal muscle homeostasis. The particular susceptibility of the kidney to drug toxicity can largely be attributed to its anatomy and function. As the filtrate moves along the complex tubular structure of each nephron, its components can be concentrated in excess of threefold in the proximal tubule, and in some cases to much higher levels (>100-fold) in the distal tubule and collecting duct. These high intratubular concentrations, together with the avid tubular uptake mechanisms, particularly in the proximal tubule, enhance intracellular concentrations. In addition, basolateral uptake of toxic agents delivered at high rates from the peritubular capillaries can contribute to intracellular accumulation. Biotransformation of

drugs to toxic metabolites also potentiates toxicity to tubular epithelial cells[4]. Furthermore, nephrotoxins can accumulate to high concentrations in the medulla as a result of the countercurrent exchange function of the medullary vasculature. The hypoxia of the medulla also increases the susceptibility of tubular cells to nephrotoxicants when the toxin results in enhanced oxygen metabolism.

One approach to the early detection of kidney injury involves defining different biomarkers that rely on the mechanisms of toxicity of each drug or drug class. However, this approach can be problematic for the many clinically useful agents for which the mechanism of toxicity is not well established. An alternative approach, to which we subscribe, involves finding a limited number of biomarkers that identify injury to primary sites in the kidney, such as the glomerulus or the proximal tubule, which together represent the major sites of toxicity related to >90% of drugs. Drugs with different mechanisms of toxicity frequently affect different parts of the kidney, as is evident from Figure 1, which shows the primary sites of nephron toxicity for various drugs. The most likely explanation for this observation is that different regions of the nephron are characterized by different transporters, metabolic characteristics, blood flow characteristics and oxygen tensions. Most drug-induced renal injuries affect the proximal tubules. Drug toxicity initially targeted to the glomerulus or more distal parts of the nephron may also cause secondary injury to proximal tubules. Detection of proximal tubule injury might thus provide a sensitive way to monitor most, but not all, toxicities. After these markers of glomerular and proximal tubule injury are established, additional ones can be added to reflect abnormalities of the distal and collecting tubules and ducts or papillary injury.

Histopathological changes in the kidney are associated with drug toxicity. These changes have been well characterized in commonly used experimental animals, and they currently remain as the ‘gold standards’ against which biomarkers from body fluids are measured. Although histopathology is the gold standard to detect renal injury, it is not without its shortcomings, even in animals where the entire organ can be examined. For example, it does not identify non–histopathology-associated types of kidney disturbances, such as either inhibition of transporters in the proximal tubule (resulting in glucosuria, aminoaciduria or hyperuricosuria) or inhibition of vasopressin action in the collecting duct (resulting in diabetes insipidus). Furthermore, a degree of subjectivity is associated with histopathological evaluation. Finally, use of histopathology invariably introduces a delay in appearance of injury; following exposure to nephrotoxicants, levels of at least some biomarkers are reported to appear before obvious changes in histology are evident.

The use of histopathology as a benchmark for kidney injury in humans is usually impractical, except in relatively rare instances when a kidney biopsy is justified. Even in such instances, however, the pathophysiology of the toxicity is associated with spatial variability in tissue injury due to vascular factors and variation in susceptibility of the tubules to injury. As biopsies usually permit only limited sampling of kidney tissue, these factors complicate the interpretation of the histopathology. Furthermore, in humans there are frequently coincident pathophysiological processes, which complicate the interpretation of biomarker data. For example, a blood or urine marker that is produced by an organ other than the kidney, which enters the bloodstream and is filtered by the kidney, can be misinterpreted as reflecting kidney injury. Increased urinary levels of a marker that is expressed by vascular or blood cells in addition to kidney tubules may reflect systemic perturbation rather than kidney injury. The strong foundation provided by detailed understanding of the sensitivity and specificity of a biomarker in various contexts of injury is thus critical to its appropriate use in animals and/or humans.

Existing biomarkers for detecting kidney injury

Two serum biomarkers, serum creatinine (SCr) and blood urea nitrogen (BUN), are commonly used to detect kidney toxicity in preclinical and clinical studies as well as in routine clinical care. Both, however, have severe limitations relating to sensitivity and specificity.

Most of the >35 different definitions of AKI in the published literature[5] rely on changes in SCr, which are insensitive for the detection of histological injury in preclinical toxicity studies, as has been demonstrated in rats, in this issue[6], as well as in humans. This is particularly true for patients with a substantial renal reserve, defined by the fact that a relatively large amount of injury can occur without producing a change in glomerular filtration rate as reflected by increases in SCr, the standard biomarker used for evaluation of kidney dysfunction. Likewise, in rodents and other animals in which drug safety experiments are conducted, with standard approaches baseline SCr levels are often at the lower end of the detectable range, and there needs to be substantial injury before SCr levels increase outside the ‘normal’ range.

Thus, in humans as well as in experimental animals, a measurable change in glomerular filtration rate (GFR) or other measures of kidney function may be evident only after considerable injury has occurred. For example, a 53% incidence of nephrotoxicity in a study involving amphotericin[7] was determined using the criterion of a doubling of SCr levels. This represents a 50% decrease in GFR if we assume creatinine production is constant. In comparison, recent definitions of AKI rely on changes in SCr of as little as 0.3 mg/dl8, representing far less than a 50% reduction in GFR in adults. These small changes in SCr are associated with significant effects on mortality9. The limitations of using SCr as a sensitive indicator of nephrotoxicity are further underscored by bearing in mind that loss of muscle mass in ill patients means that an even greater reduction in GFR is necessary to double SCr concentration. As SCr is affected not only by GFR, but also by the systemic production of creatinine and the tubular secretion of creatinine, changes in SCr concentration are not specific to tubular injury.

Serum creatinine concentration may result in a very delayed signal even after considerable kidney injury. Large changes in GFR may be associated with relatively small changes in SCr in the first 24–48 h following AKI, resulting not only in delayed diagnosis and intervention but also in underestimation of the degree of injury[10]. It is not until SCr reaches a new steady state that it becomes a reasonable measure of the new GFR. Moreover, when renal function improves, SCr underestimates GFR until a new steady state is reached. Finally, considerable variability among patients in the correlation between SCr and baseline GFR, the magnitude of functional renal reserve, and rates of creatinine synthesis means that renal injury of comparable magnitude may result in disparate alterations in creatinine kinetics and steady-state values in different individuals.

BUN is another widely used measure of renal function, but it is not a reliable measure of kidney injury because many factors may affect its concentration. BUN is freely filtered by the glomerulus, but urea is then reabsorbed to varying degrees by other parts of the nephron. Therefore, an increase in BUN can be seen with volume depletion in the absence of any tubular injury. Furthermore, increased levels of BUN can be observed if urea production is increased, as occurs with exogenous (protein supplementation) or endogenous (catabolic states or blood in gastrointestinal tract) protein loads.

The inherent flaws in SCr and BUN not only delay the recognition of nephrotoxicity in preclinical drug development but also limit the ability to monitor for drug toxicity in humans. There is also a resultant delay in the diagnosis of AKI, which prevents timely

patient-management decisions, such as withdrawal or reduction in dose of the offending agent or administration of agents to mitigate the toxicity.

Second-generation biomarkers for acute kidney injury

Several alternatives to SCr and BUN have been proposed in response to the urgent need for biomarkers that predict human nephrotoxicity in preclinical studies, allow more timely diagnosis of AKI in humans and ideally localize the injury to a specific nephron site. Although many biomarker candidates have failed to show sufficient specificity and sensitivity for clinical use, several promising candidates have emerged recently (Table 1). These include urinary kidney injury molecule- 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), cystatin C, clusterin, fatty acid binding protein–liver type (L-FABP) and osteopontin. Not only do these biomarkers have the potential to both transform the way we detect and quantify nephrotoxicity and prevent the development and entry into the market of nephrotoxic drugs, but they may also allow the continued development of potentially useful drugs that, without the help of biomarkers, would be erroneously believed to be toxic on the basis of a particular preclinical model.

It is important to consider that biomarkers for one type of kidney toxicity may not be as useful in another. A good biomarker for injury may not reliably indicate delayed repair; a biomarker that detects inflammation effectively may not be as sensitive in detecting early proximal tubule toxicity in the absence of inflammation. A biomarker of injury might not detect a functional defect, such as is observed in Fanconi syndrome or nephrogenic diabetes insipidus. And a biomarker useful in an animal model may or may not be useful in the same way in humans. Another question is whether panels of biomarkers will be more informative than a single biomarker. At first, this might seem logical because different biomarkers might be more sensitive or specific for different forms of injury. Nonetheless, if multiple biomarkers are used to detect a similar form of injury, an adjudication process will be necessary if the biomarkers suggest different outcomes.

Conclusions

Drug-induced nephrotoxicity plays an important role in the high incidence and prevalence of AKI and may serve as an important contributor to chronic renal disease. Current metrics, such as SCr and BUN, lack the sensitivity and/or specificity to adequately detect nephrotoxicity before significant loss of renal function. Better biomarkers will allow drug developers to make more informed decisions about which products to move forward in testing, the doses at which they should be used, and ways to design clinical trials that will provide clear information about product benefit and safety. Besides facilitating drug development, biomarkers shown to reliably predict kidney injury in experimental animals should eventually be evaluated for their utility in humans to promote patient safety and guide therapeutic decisions in the clinic.

The results and knowledge gained from the PSTC Nephrotoxicity Working Group and the resulting biomarker qualification process described in this issue[11] promise to enable earlier identification of nephrotoxicity in preclinical studies, provide translational markers to monitor patient responses when there is a concern about toxicity, reduce the current high rate of attrition during clinical drug development and post-marketing, prevent or reduce the entry of nephrotoxic drugs into the market, and eventually facilitate the early management of patients who suffer kidney injury.

Box 1 Ideal features of biomarkers used to detect drug-induced kidney toxicity

The PSTC Nephrotoxicity Working Group considered several criteria as key characteristics of a renal safety biomarker. These were as follows:

- Identifies kidney injury early (well before the renal reserve is dissipated and levels of serum creatinine increase)
- Reflects the degree of toxicity, in order to characterize dose dependencies
- Displays similar reliability across multiple species, including humans
- Localizes site of kidney injury
- Tracks progression of injury and recovery from damage
- Is well characterized with respect to limitations of its capacities
- Is accessible in readily available body fluids or tissues

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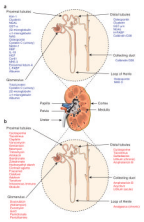

Figure 1.
The utility of biomarkers to detect injury to specific nephron segments affected by various nephrotoxicants. (**a**) Nephron segment-specific biomarkers of kidney injury. (**b**) Drugs that elicit site-specific toxicity in the kidney[12,13].

Table 1

Urinary biomarkers of kidney toxicity[12,13]

Biomarker	Model		Nephron segment	Comments
	Preclinical	Clinical		
Albumin	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI or septic AKI	Glomerulus and proximal tubule	Increased urinary excretion may reflect alterations in glomerular permeability and/or defects in proximal tubular reabsorption; increased urinary levels in the setting of fever, exercise, dehydration, diabetes, hypertension, etc., limit specificity for AKI
α-GST	Nephrotoxic AKI	Nephrotoxic AKI, septic AKI, ischemic AKI or renal transplantation	Proximal tubule	Samples require stabilization buffer for appropriate quantification; clinical data are limited
α_1-microglobulin	Nephrotoxic AKI	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule	Clinical applicability limited by lack of standardized reference levels; increased urinary levels in the setting of a number of non-renal disorders may limit specificity; and levels may predict adverse outcome (renal replacement therapy (RRT, dialysis) requirement)
β_2-microglobulin	Nephrotoxic AKI	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule	Clinical applicability limited by instability in urine
Clusterin	Nephrotoxic AKI, ischemic AKI, unilateral ureteral obstruction or subtotal nephrectomy	No AKI clinical studies to date	Proximal tubule and distal tubule	Increased urinary levels observed in rat models of tubular proteinuria but *not* glomerular proteinuria
Cysteine-rich protein	Ischemic AKI	Ischemic AKI	Proximal tubule	Urinary levels do not reflect progressive injury; levels assessed via immunoblotting (semiquantitative)
Cystatin-C	Nephrotoxic AKI	Nephrotoxic AKI, ischemic AKI or septic AKI	Glomerulus and proximal tubule	Urinary levels may predict adverse outcome (RRT requirement)
Exosomal fetuin-A	Nephrotoxic AKI or ischemic AKI	Septic AKI or ischemic AKI	Proximal tubule	Levels assessed via immunoblotting (semiquantitative); limited clinical data (n = 3)
Heart-type fatty acid-binding protein	Nephrotoxic AKI ischemic AKI	Nephrotoxic AKI or renal transplantation	Distal tubule	Increased urinary levels in the setting of heart disease may limit specificity

Biomarker	Model		Nephron segment	Comments
	Preclinical	Clinical		
Hepatocyte growth factor	Nephrotoxic AKI, ischemic AKI or unilateral nephrectomy	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule and distal tubule	Urinary levels may predict adverse outcomes (death or RRT); may play an important role in renal repair and regeneration following AKI
Interleukin-18	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule	Urinary levels may predict adverse outcomes (death)
Kidney injury molecule-1	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule	Levels may predict adverse outcome (death or RRT)
Liver-type fatty acid-binding protein	Nephrotoxic AKI, ischemic AKI or unilateral ureteral obstruction	Nephrotoxic AKI or ischemic AKI Septic AKI or renal transplantation	Proximal tubule	Levels may predict adverse outcome (death or RRT); increased urinary levels in acute liver injury may limit specificity
N-Acetyl-β-glucosaminidase	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI, septic AKI or renal transplantation	Proximal tubule	Levels may predict adverse outcome (death/RRT); decreased activity in the presence of heavy metals may limit sensitivity for AKI; and increased urinary levels in the setting of several non-renal disorders may limit specificity
Netrin-1	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI or septic AKI	Proximal tubule	Levels assessed via immunoblotting (semiquantitative); limited clinical data (n = 14)
Neutrophil gelatinase-associated lipocalin	Nephrotoxic AKI or ischemic AKI	Nephrotoxic AKI, ischemic AKI or septic AKI	Proximal tubule and distal tubule	Levels may predict severity of AKI and adverse outcome (RRT); increased levels in the setting of urinary tract infections or sepsis may limit specificity
Osteopontin	Nephrotoxic AKI, ischemic AKI or unilateral ureteral obstruction	No AKI clinical studies to date	Proximal tubule, loop of Henle and distal tubule	Increased urinary levels observed in rat models and humans following nephrotoxicity
Retinol-binding protein	Nephrotoxic AKI	Nephrotoxic AKI, septic AKI, ischemic AKI or renal transplantation	Proximal tubule	Decreased sensitivity may be observed in vitamin A–deficient states
Sodium/hydrogen exchanger isoform 3	Nephrotoxic AKI	Nephrotoxic AKI, septic AKI, ischemic AKI or renal transplantation	Proximal tubule and loop of Henle	Levels assessed via immunoblotting (semiquantitative)