Sensitive and Early Markers of Renal Injury: Where Are We and What Is the Way Forward?

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BACKGROUND

Measurement of total urinary protein, glucose, and the appearance of certain enzymes such as N-acetyl-β-D-glucosaminidase has been used in both animals and man for many years to provide insight into the onset of renal injury. However, it is clear that serum creatinine and such markers are not very sensitive and are generally only raised when acute renal injury or chronic renal injury is well established. With the coming of the “omics” age and the development of transcriptomics, proteomics, and metabonomics, it has become possible to interrogate urine samples to identify new proteins or small organic molecules appearing in urine as the result of a toxic insult or in models of renal disease. This new technology has stimulated considerable interest and effort in trying to identify novel, sensitive, and early urinary biomarkers of renal injury. A sensitive tissue marker of tubular injury, which can be used to identify or confirm the presence of epithelial cell injury even when morphological changes are minimal during the development of a new drug or pesticide or for the evaluation of biopsy material, would be welcomed by all concerned. However, history tells us that it is not one single urinary measurement, but a number of measurements that indicate that renal injury has occurred. Thus, ideally, we are looking for a number of biomarkers that together may inform us not only of the presence of injury but perhaps in which part of the nephron the injury is located.

RECENT ADVANCES IDENTIFYING NEW RENAL MARKERS

Microarray technology has been used to identify novel genes that ideally are present at low levels in normal renal tissue but are upregulated in renal tissue from rats exposed to a broad range of nephrotoxic chemicals but not in renal tissue from rats exposed to nonnephrotoxic chemicals and chemicals that caused hepatic damage. These were typically large studies conducted in pharmaceutical companies or multicenter collaborative projects where clinical chemistry, renal pathology, and gene changes were monitored in the same animals (Amin et al., 2004; Kondo et al., 2009; Thukral et al., 2005; Wang et al., 2008). These studies have identified a relatively small number of genes that are specifically altered in acute renal tubule injury. The front-runners are genes, such as osteopontin, clusterin, Glutathione S-transferase α, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (Kim-1), tissue inhibitor of metalloproteinase-1 (TIMP-1), interleukin 18 (IL-18), and cystatin C. It has now become important to validate these as protein markers in urine and to confirm or refute their selectivity and sensitivity for use in preclinical studies and in disease states in humans. A number of emerging technologies such as Luminex, Meso Scale, etc. have made it possible to measure several proteins in a single sample in a reliable and rapid way, in multiwell plates, such that large numbers of samples can be analyzed and run in a high throughput manner. Another driving force has been the regulatory agencies such as the Food and Drug Administration (FDA) in the United States and the European Medicines Agency (EMEA) asking the industry to identify biomarkers of renal injury that could be used to aid the early detection of renal injury in preclinical studies. In the United States, the International Life Sciences Institute-Health and Environmental Safety Institute (ILSI-HESI), Biomarkers of Nephrotoxicity Project Group, and the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) (both multinational groups of toxicologists from the pharmaceutical industry) have been working on this approach. In Europe, the Innovative Medicines for Europe PredTOX project is a collaborative effort by 12 pharmaceutical companies, two small and midsize enterprises,
and three universities with the aim of assessing the value of combining “omics” technologies with conventional toxicology methods to improve safety assessment of liver and kidney toxicity. In this issue of Toxicological Sciences, a manuscript by Malley et al. (2010) reported on the performance of some of the urinary proteins discussed earlier as novel markers of renal injury in preclinical toxicology studies. Basically, these authors dosed male Wistar rats with four different chemicals for 14 days, which produced varying degrees of proximal renal tubule injury assessed by histopathology without any increase in urinary glucose, protein, or elevation in serum creatinine. Measurement of urinary Kim-1, clusterin, NGAL, TIMP-1, and osteopontin after 1, 3, and 14 days showed that Kim-1 and clusterin performed well in these studies with marked increases in affected rats with receiver-operator characteristics curves giving values of 0.99 and 0.93 when correlating the increase in biomarker with the extent of histopathological damage. NGAL did not perform well, TIMP-1 was a poor indicator, and the authors were unable to measure osteopontin. These findings with Kim-1 and clusterin are supportive and consistent with data submitted by the PSTC to the U.S. FDA and EMEA who have accepted albumin, β2-microglobulin, clusterin, cystatin C, kidney injury molecule-1 (Kim-1), trefoil factor 3, and total protein as “qualified” biomarkers of renal injury in rodents (Dieterle et al., 2010; Ozer et al., 2010; Vaidya et al., 2010; Yu et al., 2010). As a consequence of the efforts of the PSTC, the FDA, and the EMEA will allow drug companies to submit the results generated using these seven urinary markers to evaluate kidney damage during animal studies of new drugs.

Kim-1 is one of the best-characterized urinary biomarkers to date in both experimental animals and humans with renal disease. In 1998, the isolation and characterization of Kim-1 were reported by Ichimura et al. (1998) from Joe Bonventre’s group at Harvard Medical School; they showed that Kim-1 was virtually undetectable in healthy kidney tissue, although it was abundantly expressed in postischemic kidneys. Kim-1 is a type I transmembrane glycoprotein with an ectodomain containing a six-cysteine immunoglobulin-like domain, two glycosylation sites, and a domain rich in threonine/serine and proline, which is characteristic of mucin-like O-glycosylated proteins. The cell surface (mature) form of Kim-1 is a 104-kDa peptide, whereas the shed, soluble form of Kim-1 that appears in urine of rodent and humans with renal injury, is about 90 kDa (Bailly et al., 2002). Kim-1 is localized in the apical membrane of dilated tubules in acute and chronic injury (Han et al., 2002; Vaidya et al., 2008; van Timmeren, van den Heuvel, et al., 2007). In ischemic injury, Kim-1 expression is most prominent in the S3 segment in the corticomedullary region, which is the part of the nephron most susceptible to ischemic injury. Kim-1 expression is also prominent in the midcortical and superficial tubules in renal disease models, where the primary insult is not directed to the S3 segment (Kramer et al., 2009; Kuehn et al., 2002). Kim-1 not only functions as a biomarker but also has predictive value for acute renal injury, e.g., Liangos et al. (2007) showed that urinary Kim-1 was predictive for adverse clinical outcome in a cohort of 201 hospitalized patients with acute renal failure. Patients within the highest Kim-1 quartile had a 3.2-fold odds ratio for dialysis or hospital death compared with patients in the lowest quartile. Kim-1 has also been shown to have prognostic significance in transplant recipients; renal Kim-1 expression is more sensitive than histology for detecting early tubular injury in human allografts (van Timmeren, Vaidya, et al., 2007; Zhang, Rothblum, et al., 2008).

In animal models of acute renal injury, Kim-1 gene and protein products are upregulated 3 h after experimental renal ischemia-reperfusion injury (Vaidya et al., 2006). While chemically induced renal injury produced by cisplatin, folic acid, S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (Ichimura et al., 2004), and other nephrotoxic agents (Zhang, Brown, et al., 2008; Zhou et al., 2008) resulted in up regulation of Kim-1. Thus, the selective Kim-1 expression by injured proximal tubule cells provided a strong impetus for using Kim-1 as a biomarker of damage. Kim-1 is measured in urine using microsphere-based Luminex xMAP technology with monoclonal or polyclonal antibodies raised against rat or human Kim-1 ectodomain. Studies have shown that the ectodomain of Kim-1 is stable at room temperature and hence can be quantified in 24-h urine where a sample size of 30 µl is needed for measurement. The lower limit of detection is 4 pg/ml and mean urinary Kim-1 excretion in control subjects is 58 (42–74) ng/day, whereas nondiabetic patients, with proteinuria of about 3.8 g/day, excreted 1706 ± 498 ng Kim-1/day (Waanders et al., 2009). Recently Kim-1 dipsticks (RenaStick) have been developed as a rapid diagnostic assay for use in the clinic or at the bedside, providing a sensitive assay of urinary Kim-1 within 15 min (Vaidya et al., 2009). In summary, Kim-1 appears to play a role in the pathogenesis of tubular cell damage and repair in experimental and human kidney disease. Elevated urinary Kim-1 levels are strongly related to tubular Kim-1 expression in both animals and man, indicating that Kim-1 is a very promising biomarker for the presence of tubule-interstitial pathology and damage.

WHERE DO WE GO FROM HERE?

First, there is a need for many of these other small peptides that have been proposed as biomarkers to be validated in experimental animals and assessed in man. Kim-1 is way ahead of some of the other markers at this time, but there is still an urgent need for the detection and monitoring of kidney injury in both the acute and the chronic disease setting. More proteins are likely to be detected in urine in various disease states, including renal cell carcinoma, which is of tubular origin, in the future. High-resolution two-dimensional gel electrophoresis of the human urinary proteome has yielded nearly 1400 distinct protein spots (Pieper et al., 2004). Protein profiling using surface-enhanced laser desorption/ionisation-time of
flight-mass spectrometry and other analytical techniques also holds out promise to provide new insights for biomarker identification and validation in urine (De Bock et al., 2010). However, one of the most exciting and rapidly expanding fields is microRNAs (miRNAs). miRNAs are regulatory RNAs that act as posttranscriptional repressors by binding the 3’ untranslated region of target genes and have been implicated in diverse biological and pathological processes. Studies with conditional Dicer knockout mice have revealed critical roles for miRNA in orchestrating kidney development and maintaining the structural and functional integrity of the renal collecting system and glomerular barrier. Expression profiling has provided a reasonably clear picture of miRNAs present in normal kidney and pointed to individual miRNAs that may serve specific functional roles. Specific miRNAs have been implicated in pathways linked to cystic kidney disease (miR-15a) and Wilms’ tumor (miR-17-92). Several miRNAs are upregulated by transforming growth factor β-1 in models of diabetic nephropathy; some promote matrix deposition (miR-192 and miR-377) or epithelial to mesenchymal transition (miR-200 and miR-2005) (Saal and Harvey, 2009). More interesting is the finding of miRNAs in urinary exosomes (Melkonyan et al., 2008; Simpson et al., 2009), which potentially could serve as disease biomarkers in humans and markers of renal injury in experimental animals. In support of this notion, miRNA-208, revealed to be specific for the heart, was shown to increase in the plasma of rats treated with isoproterenol-induced myocardial injury (Ji et al., 2009).

CONCLUSIONS

Considerable progress has been made in the last decade in identifying biomarkers of renal tubular injury in experimental animals and more importantly in humans with acute renal injury and in some cases chronic renal injury. The biomarker Kim-1 is the most validated to date, but other proteins are being assessed, some of which have been qualified as biomarkers of renal injury for preclinical studies by regulatory agencies. Improved analytical techniques and studies on miRNA excretion in urine are exciting prospects for adding to our biomarker armory.

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