

New Approaches for Detecting Thresholds of Human Nephrotoxicity Using Cadmium as an Example

Patricia W. Mueller,¹ Robert G. Price,² and William F. Finn³

¹Centers for Disease Control and Prevention, Atlanta, GA 30341 USA; ²Division of Life Sciences, King's College, London, United Kingdom; ³University of North Carolina School of Medicine, Chapel Hill, NC 27599 USA

Damage to the kidneys is one of the primary toxic actions of metals. Nephrotoxic substances not only cause renal disease directly, but they can also destroy renal reserve capacity, potentially placing those people with additional risk factors, such as diabetes, hypertension, cardiovascular disease, and genetic predispositions, at greater risk. To detect nephrotoxicity in people at a stage where intervention can be effective, sensitive methods are needed. One of the major advantages of using sensitive biomarkers of renal damage is that people who may be particularly susceptible to renal damage can be identified early, at a reversible stage of damage, and the progression to end-stage renal disease may be halted or delayed. Various categories of tests can be used to detect effects of nephrotoxic substances on the kidney. Through the use of biomarkers of damage to various parts of the nephron, U.S. and European studies have both shown a similar pattern of damage among men occupationally exposed to cadmium. These studies indicate various thresholds of renal effects, which researchers suggest represent a cascade of progressively severe damage to the kidney. Research into new biomarkers of damage caused by exposure to nephrotoxic substances centers around mechanisms of cell death, including necrosis and apoptosis; mechanisms of cell growth, regeneration, and proliferation, including factors that control cell cycle, influence gene expression, and modulate nucleic acid synthesis; and genetic factors that increase susceptibility to renal disease. Examples of types of candidate biomarkers include cytokines, lipid mediators, growth factors, transcription factors and protooncogenes, extracellular matrix components (collagen, glycoproteins, and proteoglycans), and cell adhesion molecules. Research into new categories of biomarkers may provide additional insights into the mechanisms of damage caused by nephrotoxins. *Key words:* biomarkers, cadmium, human, kidneys, lead, mercury, metals, nephrotoxicity, solvents. *Environ Health Perspect* 106:227–230 (1998). [Online 23 March 1998] <http://ehpnet1.niehs.nih.gov/docs/1998/106p227-230mueller/abstract.html>

Damage to the kidneys is one of the primary toxic actions of metals, and nephrotoxicity by heavy metals has been the focus of much research. In the United States, renal disease exacts a heavy burden, both in human and economic terms. In 1990, for example, approximately 165,000 people received chronic dialysis therapy for end-stage renal disease (ESRD), and another 9,800 people received renal transplants at a cost to the federal government, alone, of \$5.22 billion (1). Nephrotoxic substances not only cause renal disease directly, but they can also destroy renal reserve capacity, potentially placing people with additional risk factors, such as diabetes, hypertension, cardiovascular disease, and genetic predispositions, at greater risk.

To detect nephrotoxicity in people at a stage where intervention can be effective, sensitive methods are needed. The two kidneys each contain 1.0–1.3 million independent nephrons. Possibly one-third of the nephrons could be lost to a chronic disease process or nephrotoxicity without a noticeable reduction in the whole-kidney glomerular filtration

rate. If the remaining two-thirds of the nephrons hypertrophied so that the single nephron filtration rate increased by 50%, the whole-kidney glomerular filtration rate would remain the same. As a result, the kidney can suffer considerable damage before losing sufficient function to modify the normal clinical indicators of renal disease, such as the serum creatinine concentration. Approximately 50% or more of renal capacity can be lost before serum creatinine levels become abnormal (2) and the disease is detectable clinically. This lack of sensitivity of the normal clinical indicators has led to the use of more sensitive tests to detect damage to various parts of the nephron. These tests include indicators of glomerular selectivity, indicators of tubular protein reabsorption capability, and indicators of turnover of tubular tissue. Active research into new methods of further defining early renal damage is ongoing. These new methods frequently provide a means of quantifying and describing the lowest measurable human effects of nephrotoxic substances such as metals, and therefore allow the

determination of the lowest limit of these effects in humans. New methods also potentially allow individuals who are particularly susceptible to the effects of nephrotoxic substances to be identified. A battery made up of a combination of different types of tests can aid in the quantitative description of the pattern of damage of a nephrotoxin and also allow for the determination of various thresholds of damage. The detection of renal damage at a reversible stage is necessary before effective preventive measures can be taken to halt the progress of damage to the irreversible stage.

Categories of Tests

Various categories of tests can be used to detect effects of nephrotoxic substances on the kidney. Seven categories of tests were identified at the first U.S./European Union Workshop, "Urinary Biomarkers to Detect Significant Effects of Environmental Occupational Exposure to Nephrotoxins" (3):

1. Clinical indicators of renal disease: a decrease in the glomerular filtration rate (GFR) as determined by inulin, iothalamate, or creatinine clearance; an increase in serum creatinine concentration; the presence of dip-stick positive proteinuria; elevated serum cystatin C levels.
2. Early indicators of glomerular damage: elevated albumin or transferrin excretion without an increase in low molecular weight (LMW) protein excretion; the

Address correspondence to P.W. Mueller, Centers for Disease Control and Prevention, MS F50, 4770 Buford Highway NE, Atlanta, GA 30341-3724 USA. Funding for the Joint United States/European Union Workshop, "Urinary Biomarkers to Detect Significant Effects of Environmental and Occupational Exposure to Nephrotoxins," was provided by the National Center for Environmental Health, Centers for Disease Control and Prevention, the European Commission and its Environmental Research Program, the Agency for Toxic Substances and Disease Registry (from the Comprehensive Environmental Response, Compensation, and Liability Act Trust Fund), the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute for Environmental Health Sciences, and Bayer Corporation.

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- excretion of extracellular matrix proteins of glomerular derivation.
3. Early indicators of damage to the tubular protein absorption capability: elevated excretion of retinol-binding protein (RBP), β_2 -microglobulin (β_2m , unstable in some urine samples), or Clara cell protein (a more sensitive indicator in women).
 4. Indicators of direct release of tubular tissue into urine: increased urinary excretion of *N*-acetyl- β -D-glucosaminidase (NAG, lysosomal), intestinal alkaline phosphatase (IAP), alanine aminopeptidase (AAP), and tubular antigens.
 5. Early indicators of damage to the distal tubule: loss of urine-concentrating ability, as indicated by decreased urine osmolality; increased excretion of Tamm-Horsfall protein and π glutathione *S*-transferase; changes in epidermal growth factor (EGF) excretion.
 6. Early indicators of interstitial damage: none available.
 7. Changes in prostaglandin synthesis.

Some of these tests have been used in human studies (e.g., GFR, serum creatinine, osmolality, urine albumin, RBP, NAG, AAP, IAP, and β_2m), and their behavior has been characterized in response to exposures to different nephrotoxic metals (4-9) as well as other nephrotoxic substances. Other tests have not been used in as many human studies of nephrotoxicity and are not as well characterized.

Thresholds of Effects: Cadmium

The renal toxicity of cadmium has been studied more extensively than that of other heavy metals. Through the use of biomarkers of damage to various parts of the nephron, a pattern of damage is emerging that is consistently observed now both in U.S. and European studies of men occupationally exposed to cadmium (4-10). These studies indicate various thresholds of renal effects, which European investigators have suggested represent a cascade of progressively severe damage to the kidney (3,11). The observed thresholds are based on a significant increase in the prevalence of abnormal values. Test values become abnormal in the most susceptible individuals first, and with increasing cadmium exposure the number of individuals with abnormal values increases. These thresholds are discussed below.

The lowest observed effect is indicated by a significant prevalence of abnormal 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) and sialic acid levels in urine at an approximate urine cadmium concentration of 2.4 nmol Cd/mmol creatinine (2.4 μ g Cd/g creatinine) (10). Proteins such as albumin and transferrin originate in the plasma and

are filtered to a limited degree through the glomerulus, and the protein that is filtered is primarily reabsorbed by the tubules. When tubular protein reabsorption is normal, as indicated by normal excretion of LMW proteins such as RBP and β_2m , elevations in the excretion of albumin and transferrin indicate changes in glomerular selectivity. Significant elevations in the excretion of albumin and transferrin occur at urine cadmium concentrations from 3.6 to 4.2 nmol Cd/mmol creatinine (3.6-4.2 μ g Cd/g creatinine) (5,10).

Significantly increased excretion of indicators of direct release of tubular tissue, such as brush-border antigens, NAG, IAP, and AAP, occurs at urine cadmium concentrations between 3.7 and 6.3 nmol Cd/mmol creatinine (3.7 and 6.3 μ g Cd/g creatinine) (5,10). Increased excretion of the LMW proteins RBP or β_2m is a sensitive indicator of damage to the tubules and loss of tubular protein reabsorption capability (3). These LMW proteins are normally freely filtered through the glomerulus and primarily reabsorbed by the tubules. Significant elevations in the excretion of these proteins occur at urine cadmium levels between 6.5 and 11.5 nmol Cd/mmol creatinine (6.5 and 11.5 μ g Cd/g creatinine) (5,10). Increased excretion of Tamm-Horsfall glycoprotein (at 7.0 nmol Cd/mmol creatinine or μ g Cd/g creatinine), which has been suggested as an indicator of distal tubular damage, and increased excretion of glycosaminoglycans (at 11.5 nmol Cd/mmol creatinine or μ g Cd/g creatinine) also occur in this same urine cadmium range (10).

These thresholds are based on studies of occupational exposures. At least three studies of environmental exposures have shown that cadmium causes elevated biomarker test results at lower cadmium levels than among occupationally exposed workers (10,12,13). Although the health significance of these findings has not been determined, one explanation for the difference is that the general population may contain more susceptible people than a healthy working population (10).

The relatively few studies of women exposed to cadmium in which the data were analyzed separately by gender indicate a different pattern of damage among young women than among occupationally exposed men, with the damage becoming apparent in women primarily after the age of menopause (5,14-17). Another difference between men and women was reported in a Swedish study of workers exposed to cadmium in a battery factory. In this study, researchers found that the percentage of workers with kidney stones was

lower among women (2.1%) than among men (12.0%) (18). In a recent study in Japan of people 56-71 years old (past the mean age of menopause for women) who had been environmentally exposed to cadmium, no difference between men and women in the incidence or severity of renal tubular dysfunction was found (19). In another Japanese study, a 15-year follow-up study of people initially \geq 50 years old showed that men and women had similar prevalences of high RBP excretion (20).

Health Significance of Abnormal Test Values

The clinical significance of three categories of tests has been studied to varying degrees. Increased albumin excretion has been epidemiologically associated with, and is predictive of, renal disease among people with insulin-dependent diabetes mellitus (21). Among people with non-insulin-dependent diabetes mellitus, those with hypertension (without diabetes), and those in the general population (the Framingham Study), increased albumin excretion and proteinuria are primarily associated with excess cardiovascular disease, mortality, and renal disease (21). Persistent high-grade proteinuria is associated with a progressive loss of renal function, and once present may, by itself, contribute to the secondary development of tubulointerstitial injury. Elevated albumin excretion has also been shown to be a sensitive, although not specific, indicator of analgesic nephropathy (22). Appropriate long-term studies of the clinical significance of the elevations in albumin excretion associated with metal exposures have not been done.

The second category of tests for which long-term studies are available is that of LMW proteins. Roels and co-workers have conducted long-term studies of the implications of elevated LMW proteinuria among cadmium workers and found that the LMW proteinuria that occurs when the cadmium level in urine exceeds 10 nmol Cd/mmol creatinine (10 μ g Cd/g creatinine) is irreversible (10,23) and that it is associated with both an exacerbation of the age-related decline in the glomerular filtration rate based on estimates from serum β_2m levels (10,24) and a reduction of protein-induced short-term hyperfiltration (filtration reserve capacity) (10,25). Bernard [see Bernard et al. (11)] suggested four thresholds of LMW proteinuria (RBP or β_2m): 1) <300 μ g/g creatinine should be regarded as normal, 2) 300-1,000 μ g/g creatinine indicates incipient cadmium tubulopathy (possibly reversible after removal from exposure) with no change in the GFR, 3) 1,000-10,000 μ g/g creatinine

indicates irreversible tubular proteinuria, which may lead to an accelerated decline of the GFR with age (GFR is normal or slightly impaired), and 4) $>10,000 \mu\text{g/g}$ creatinine indicates overt cadmium nephropathy, usually associated with decreased GFR. In a recent Swedish study, researchers reported associations between abnormal $\beta_2\text{m}$ excretion and a steeper dose response between cadmium exposure and renal calculi (kidney stones) (18). In one Japanese study of environmental cadmium exposure, investigators found relationships between an elevated fractional excretion of $\beta_2\text{m}$ and the results of a number of clinical tests, including increases in the fractional excretion of urate, calcium, sodium, and chloride and decreases in fractional phosphate reabsorption and creatinine clearance (19). In a 15-year Japanese follow-up study of a different environmental exposure to cadmium, investigators found an association between RBP excretion $\geq 4 \text{ mg/l}$ and an increased risk of death from cardiovascular disease and from renal disease among both men and women and an increased risk of diabetes mellitus among women (20).

The third category of tests for which data on clinical significance exist is that of enzymes or renal antigens, the origin of which is primarily the renal tubule itself rather than plasma. Membrane-bound proteins are released into the urine as a result of necrosis, apoptosis, and regenerative processes, and these regenerative processes can take different forms depending on whether the damage is acute or chronic (26). Changes in enzyme excretion can relate to regulation of exocytotic and endocytotic transport. Levels of various enzymes have been shown to be elevated in animals in conjunction with changes in renal function and exfoliation of renal tubular cells induced by administration of mercuric chloride (27,28). In humans, a model of the response of these types of proteins to nephrotoxicity is the response to cisplatin chemotherapy. Cisplatin causes cystic alterations, tubular loss, inflammation, and multiple areas of hyperplasia in the kidneys of rats (29). Goren et al. (30) have shown that NAG and AAP activities increase in humans 3 days after receiving cisplatin therapy, just as tubular necrosis appears in rats 3 days after exposure to cisplatin. Goren and co-workers also observed a chronic baseline rise in NAG activity in children following the activity spikes that occur with each cisplatin dose. Increased levels of NAG and AAP activity have also been associated with loss of renal tubular secretion function. They found that NAG and AAP activities were associated with significant increases in the half-life of methotrexate, a drug that is

substantially secreted by the tubules of patients who have received cisplatin, and that NAG activity, in particular, was predictive of impaired renal excretion of methotrexate (31).

There are few, if any, data on the health significance of changes in other categories of tests. A more definitive picture of the relationship between various biomarkers of nephrotoxicity and the development of renal disease awaits more extensive prospective studies and studies using clinical models of nephrotoxicity.

Potential New Biomarkers

Research into new biomarkers of damage caused by exposure to nephrotoxic substances focuses on several major areas. These areas include research into mechanisms of cell death, including necrosis and apoptosis; research into mechanisms of cell growth, regeneration, and proliferation, including factors that control cell cycle, influence gene expression, and modulate nucleic acid synthesis; and research into factors that increase susceptibility to renal disease, including preexisting conditions and genetic factors (1,3,32).

Examples of types of candidate biomarkers include cytokines, lipid mediators, growth factors, transcription factors and protooncogenes, extracellular matrix components (collagen, glycoproteins, and proteoglycans), and cell adhesion molecules (32). Some of these candidate markers are beginning to be examined in human studies. Excretion of fibronectin has been examined in relation to exposure to three heavy metals (cadmium, lead, and mercury), with no significant changes in excretion seen in the exposed groups (7-9).

Excretion of prostanoids [6-keto-PGF $_{1\alpha}$, PGE $_2$, PGF $_{2\alpha}$, and thromboxane B $_2$ (TXB $_2$)] was examined in the same three heavy metal exposure studies (7-9), with different results for exposures to mercury, lead, and cadmium. PGI $_2$ (measured as the stable urinary derivative 6-keto-PGF $_{1\alpha}$) and PGE $_2$ are vasodilators and contribute to mesangial relaxation, whereas PGF $_{2\alpha}$ and TXB $_2$ are vasoconstrictors and/or contribute to mesangial contraction (3). In the case of mercury exposure, the excretion of PGE $_2$, PGF $_{2\alpha}$, and TXB $_2$ were all significantly lower among the exposed workers. Among workers exposed to lead, the excretion of the vasodilator PGI $_2$ was significantly lower than in controls, whereas the excretion of the vasoconstrictor TXB $_2$ was significantly higher. Cadmium exposure among workers was associated with increased excretion of both 6-keto-PGF $_{1\alpha}$ and PGE $_2$ and no change in the excretion of PGF $_{2\alpha}$ or TXB $_2$. These findings may have mechanistic implications

for the controversial question of how metal exposure, especially exposure to lead, affects blood pressure (8).

In rats, renal excretion of EGF has been shown to decrease immediately in response to the release of ischemia in the rat (33). Human studies of EGF excretion have been largely limited to people with diabetes and have generally shown that under near-normal glycemic conditions, EGF excretion correlates with the GFR (34), and EGF excretion may decrease with progressive diabetic complications (35). Under hyperglycemic conditions, however, there were indications that EGF excretion may increase (35). EGF has been suggested as an indicator of distal tubular damage, which may be more closely related to changes in the GFR than some other classes of markers (32).

Another distal tubular marker that has been proposed recently is π glutathione S-transferase (π GST). Whereas α GST is found primarily in the proximal convoluted tubules of the kidney, π GST is found in the distal convoluted tubules, the thin loop of Henle, and the collecting ducts (36,37). Damage to the epithelial cell membranes in these regions results in increased excretion of this isoenzyme (37). It has been suggested that excretion of different isoforms of GST may distinguish between certain pathological processes in the kidney (38).

The best approach for validation of new biomarkers is an issue that received considerable discussion in the U.S./EU workshop. Long-term follow-up studies of cohorts occupationally exposed to cadmium was suggested and discussed (11). Although this might be feasible with existing cohorts, three considerations were raised with regard to this type of study: ethical concerns because of the need to remove workers from exposure, which might also reverse effects; the "healthy worker effect," which limits extrapolation of the conclusions, and the increasing difficulty of finding highly exposed workers because of lower exposure levels. The final recommendation of the workshop was to examine the use of clinical models of nephrotoxicity to evaluate new biomarkers and to study the development of renal disease.

Conclusions

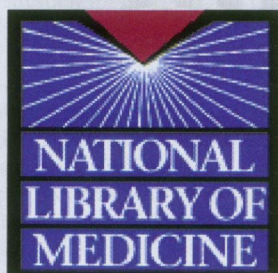
It is now possible to draw a more accurate picture of early damage to the human kidney by nephrotoxic substances such as metals. It is possible to define levels of body burden or exposure in humans that are associated with various levels of damage indicated by different types of biomarkers. Research into new categories of biomarkers may provide additional insights into the

mechanisms of damage caused by nephrotoxins. Although a variety of biomarkers are frequently used when studying nephrotoxic substances, as new information becomes available on individual toxicants and particular combinations of toxicants, smaller, more focused batteries of biomarkers can be used to describe the characteristic pattern of damage.

One of the major advantages of using sensitive biomarkers of renal damage is that people who may be particularly susceptible to renal damage may be identified early, when damage to their kidneys is reversible and the progression to end-stage renal disease may be halted or delayed (39). In addition, during the next 10 years, genetic factors may be found that may be associated with susceptibility to various types of toxicity, including renal toxicity.

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