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Renal dysfunction induced by cadmium: biomarkers of critical effects

Alfred Bernard
Unit of Toxicology, Catholic University of Louvain, 30.54 Clos Chapelle-aux-Champs, 1200 Brussels, Belgium (E-mail : bernard@toxi.ucl.ac.be)

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Abstract

Cadmium (Cd) is cumulative poison which can damage the kidneys after prolonged exposure in the industry or the environment. Renal damage induced by Cd affects primarily the cellular and functional integrity of the proximal tubules, the main site of the renal accumulation of the metal. This results in a variety of urinary abnormalities including an increased excretion of calcium, amino acids, enzymes and proteins. These effects have been documented by a large number of studies conducted during more than two decades in experimental animals and in populations environmentally or occupationally exposed to Cd. There is now a general agreement to say that the most sensitive and specific indicator of Cd-induced renal dysfunction is a decreased tubular reabsorption of low molecular weight proteins, leading to the so-called tubular proteinuria. β_2-microblobulin, retinol-binding protein and α_1-microglobulin are the microproteins the most commonly used for screening renal damage in populations at risk. Tubular dysfunction develops in a dose-dependent manner according to the internal dose of Cd as assessed on the basis of Cd levels in kidney, urine or in blood. Depending on the sensitivity of the renal biomarker and the susceptibility of the exposed populations, the thresholds of urinary Cd vary from 2 to 10 μg/g creatinine. The thresholds associated with the development of the microproteinuria, the critical effect predictive of a decline of the renal function, is estimated around 10 μg/g creatinine for both occupationally and environmentally exposed populations. Much lower thresholds have been reported in some European studies conducted on the general population. These low thresholds, however, have been derived from associations whose causality remains uncertain and for urinary protein increases that might be reversible. Cd-induced microproteinuria is usually considered as irreversible except at the incipient stage of the intoxication where a partial or complete reversibility has been found in some studies.

Introduction

Cadmium (Cd) is one of the most heavily cumulative toxins with an estimated half-life of more than 15 years in man. During the 20th century, the Cd body burden of the general population of most industrialised countries has increased as a result of uncontrolled industrial emissions in the past. Although emission levels have considerably decreased and a decline of the human exposure is likely to occur in the near future, there are still populations excessively exposed to the metal in the industry and in some polluted areas.

The two main storage organs of Cd are the kidney and the liver which contain about 50% of the total body burden. The highest concentrations of Cd are found in the kidney which is considered as the critical organ whatever the source and portal of entry of Cd. The renal accumulation of Cd takes place mainly in the proximal tubules where from a certain stage the metal gives rise to a dose-dependent toxicity. Other segments of the nephron can also be affected but usually at more advanced stage of the intoxication than the proximal tubules.

Research conducted during the last decades in occupationally or environmentally exposed populations have largely documented the constellation of renal effects that can be induced by Cd as well as the thresholds of body burden from which these effects

are likely to occur. These studies have also explored a wide variety of potential biomarkers of nephrotoxicity to gain insight into the mechanisms of Cd toxicity and to identify sensitive and easily applicable markers for the health surveillance of exposed populations.

We here summarize the current state of knowledge about these biomarkers by focusing on the low-molecular-weight urinary proteins which have become now an integral part of the health surveillance programs of populations at risk.

Biomarkers of cadmium nephropathy

A wide range of potential biomarkers of Cd nephrotoxicity have been investigated in subjects exposed to Cd in the industry or the environment. Studied markers, usually measured in urine, included kidney-derived antigens or enzymes, plasma-derived proteins reflecting the permeability of the glomerular filter or the protein reabsorption capacity of proximal tubules and various small molecules normally reabsorbed by the tubules (calcium, amino acids) (Bernard, 1996; Wu *et al.* 2001). Although these markers have provided valuable insight into the mechanisms of Cd toxicity and the critical levels of exposure, few of them have proved sufficiently sensitive and reliable to be used as routine screening tests. Among these, the determination of molecular weight proteins (i.e. molecular weight <40 kDa) in urine is now recognised as the most useful approach for the early detection of Cd nephropathy. Because in healthy subjects the tubular reabsorption of these small proteins is almost complete (more than 99.9%), a small decrement in the tubular reabsorption capacity produces a marked increase of the urinary excretion of microproteins such as β_2-m or RBP which in most advanced cases of Cd nephropathy can reach levels more than 1000 times above normal. The urinary β_2-m test which was introduced more than 30 years ago is still the most widely used method. The test is sensitive and can be applied using commercially available methods. Its only drawback is that β_2-m is unstable in acidic urine (pH < 5.6), requiring thus a careful control of the urinary pH. In practice, unless the patient has been given bicarbonate before urine collection, a loss of the β_2-m is unavoidable in 10 to 30% of urine samples (Bernard 1996b). To overcome this limitation, the urinary retinol-binding protein (RBP) test was proposed in the early 80s since, while being equally sensitive as urinary β_2-m, RBP presents the advantage of a much greater stability in acid urine, necessitating specific no particular precaution with respect to the pH (Bernard *et al.* 1981). Another low-molecular protein also very stable in urine is alpha$_1$-microglobulin but according to some studies this protein might be less sensitive as marker of tubular dysfunction and possibly also less specific because of its larger molecular weight (26 kDa) (Bernard, 1996b). The human Clara cell protein - CC16 or protein 1- can also be used to screen tubular dysfunction but a post-renal secretion from the prostate reduces its specificity and sensitivity in men. In women, by contrast, urinary CC16 shows an unique sensitivity to Cd exposure, allowing to detect subtle defects of the proximal tubule that pass unseen with other proteins (Bernard *et al.*, 1994).

Screening of cadmium renal dysfunction

All biomarkers of Cd nephrotoxicity are based on the response of the kidneys to the progressive accumulation of Cd. As a direct measurement of Cd stored in kidneys by a non-invasive technique (e.g. by neutron activation) is rarely feasible, most studies have used the concentrations of Cd in urine or in blood as surrogate indicators of the Cd body burden. Owing to intrinsic differences in their sensitivity or to the sequential involvement of specific sites of the nephron, biomarkers of nephrotoxicity become abnormal from different levels of Cd exposure or body burden. This variable response is reflected by the thresholds of urinary Cd which can range from less than 2 μg/g creatine for the onset of early biochemical alterations (e.g. increased NAG excretion) to 10 μg/g creatinine for the development of the classic tubular proteinuria. From the numerous studies conducted during the last decades, a consensus has emerged that the renal effect of Cd to consider as critical for deriving safe exposure levels is an increased urinary excretion of the low-molecular-weight proteins β_2m or RBP. This is indeed the only indicator or endpoint which has been shown to be predictive of an accelerated decline of the renal function with age.

Dose-response relationships for the development of this tubular proteinuria have been established in several studies on occupationally or environmentally exposed populations. There are two important points which need to be addressed when deriving thresholds of Cd toxicity from these relations. First, a reliable derivation of thresholds is possible only when dose-response relations are established in subjects currently

Table 1. Interpretation of elevated values of urinary β_2-microglobulin (β_2-m) and retinol-binding protein (RBP)

β_2-m or RBP in urine (μg/g cr)	Significance
<300	Normal value
300–1000	Incipient Cd tubulopathy (possibility of some reversibility after removal of exposure if urinary Cd is not too high i.e. below 20 μg/g cr)
1000–10,000	Irreversible tubular proteinuria that may accelerate the decline of GFR with age. At this stage GFR is normal or slightly impaired.
>10,000	Overt Cd nephropathy usually associated with a decreased GFR

Modified from Bernard, 1996a.

exposed to Cd or examined not too late after their removal from exposure. As illustrated in Figure 1, the removal from Cd exposure is necessarily associated with a progressive decrease of Cd levels in blood and urine, which shifts the dose-response curves towards lower levels of Cd and thus results in an underestimation of the thresholds of toxicity. The second important issue to consider is that circulating Cd is not free but largely bound to proteins some of them like metallothionein, follow the same glomerular-tubular reabsorption pathway as low molecular weight proteins used as to detect tubular dysfunction. Conceivably thus, an impairment of tubular function unrelated to Cd could enhance the urinary excretion of both protein-bound Cd and β_2-m or RBP used as markers. This could generate non causal associations between Cd and low molecular weight proteins in urine, reflecting the dependence of each other on the ability of proximal tubules to reabsorb proteins. One simple way to ascertain the causality of observed relations is to check that dose-effect/response relationships also emerge with the concentration of Cd blood, an internal dose marker, which in the absence of renal insufficiency, can be considered as independent of low molecular weight in urine. This verification has been done for instance in the recent survey conducted in China (Nordberg *et al.* 2002; Jin *et al.* 2002). Statistically significant dose-effect/response relationships were observed with blood Cd as well as with urinary Cd, corresponding undoubtedly to causal associations. In workers exposed to Cd, most studies concord also in saying that the threshold of urinary Cd associated with an increased risk of tubular proteinuria lies around 10 μg/g creatinine, an estimate in agreement with the critical level of Cd in renal cortex (Bernard *et al.* 1992). Recent studies in Cd-polluted areas of China and Japan have also concluded that the risk of developing a tubular proteinuria increases in men or women when CdU exceeds a threshold around 10 μg/g creatinine (Nordberg *et al.* 2002; Ikeda *et al.* 2003). Some population-based studies, namely those conducted in Europe (Cadmibel and OSCAR studies) have reported much lower thresholds of urinary Cd for the risk of tubular proteinuria in the general population (Buchet *et al.* 1990; Jarup *et al.* 2000). In a follow-up of the Cadmibel study, renal effects associated with low levels of urinary Cd were however found to be reversible and not predictive of an accelerated ageing of the kidney (Hotz *et al.* 1999). Since in these studies no dose-response relationships were found when using Cd in blood as exposure indicator, one cannot formally exclude, as explained above, the possibility of non-causal associations due to the dependence of both Cd and low-molecular weight proteins on the reabsorption capacity of proximal tubules.

Recommendations for health surveillance

In health surveillance, the emphasis must be placed on measures of primary prevention in order to maintain the levels of Cd in the environment or in foodstuffs as low as possible. The efficacy of these measures can be controlled by measuring the metal in urine or blood of populations or groups at risk and usually a periodic screening for renal dysfunction is implemented only when excessive levels of Cd are found in urine or blood. For these reasons and also to achieve the best cost-benefit ratio, screening tests for tubular proteinuria are always used in combination with a

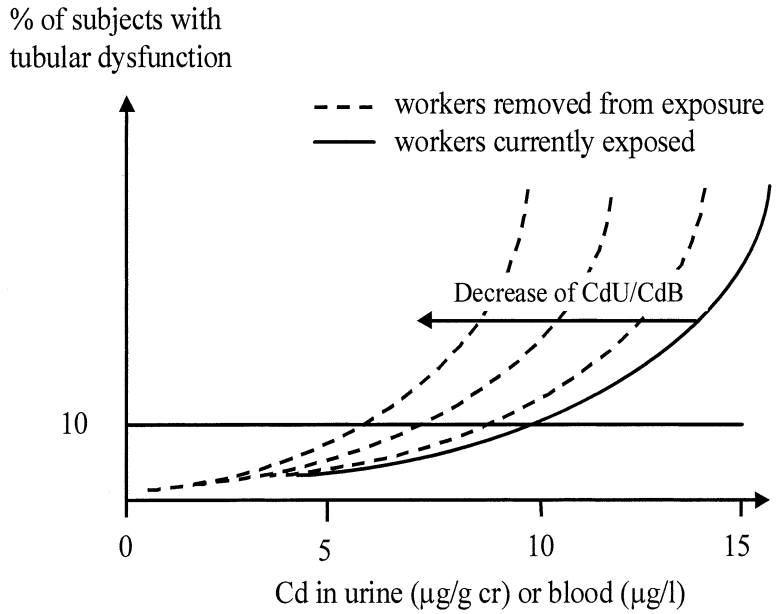

Fig. 1. Schema illustrating the shift in the thresholds of Cd in urine or blood when dose-response relations are derived from data obtained in currently exposed subjects or in subjects removed from exposure and progressively losing their Cd.

biomonitoring programme measuring Cd in urine and blood. This combination is also important for the interpretation of the results since a persistent increase in the urinary excretion of β_2-m or RBP, after exclusion of other causes, can be ascribed to Cd only when it is associated with an increased Cd body burden. In subjects with no occupational or environmental exposure, concentrations of Cd in urine are normally below 2 μg/g creatinine. Urinary Cd levels in the range of 2 and 5 μg/ g creatinine are the signs of an increased body burden resulting from an environmental or occupational exposure. Exceptionally, such levels can also be found in heavy smokers. At this stage, a periodical screening of tubular dysfunction is usually not recommended. When Cd in urine reaches values between 5 and 10 μg/g creatinine, the risk of developing a tubular proteinuria remains unlikely, except perhaps in particularly vulnerable subjects or in subjects who have been exposed in the past and progressively loose their body burden of Cd (Figure 1). For this reason, a screening for tubular proteinuria is normally recommended in all subjects who persistently show an urinary Cd above 5 μg/g creatinine, a level corresponding to the occupational exposure limit in most industrialised countries. When the concentration of Cd in urine exceeds 10 μg/g creatinine, the risk of developing a tubular proteinuria is well established. This risk increases almost linearly with the urinary Cd concentration from an expected prevalence of tubular proteinuria around 10% for CdU values slightly above 10 μg/g creatinine to more than 20% when CdU values exceed 20 μg/g creatinine.

As to the prognosis of the Cd-induced tubular proteinuria, only a persistent increase over intervals of months or years may be the sign of irreversible degenerative changes likely to compromise the renal function. Once the persistent character of the increase has been confirmed, the medical relevance of the tubular proteinuria depends primarily on the magnitude of deviation from normal. In that respect, four stages can be distinguished (Table 1) between the incipient tubulopathy to the overt nephropathy with a decreased renal function.

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