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Controlling the risk of nephrotoxicity in men occupationally exposed to inorganic mercury, lead, or cadmium through monitoring biomarkers of exposure

by

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Abstract

A successful prevention of renal diseases occurring in occupational exposure to toxic heavy metals such as mercury (Hg), lead (Pb), or cadmium (Cd) largely relies on the capability to detect nephrotoxic effects at a stage where the renal effects are still reversible or at least not yet compromising the kidney function. The knowledge of dose-effect/response relations has been instrumental in setting adequate surveillance strategies to control nephrotoxic effects of these metals through a "biological monitoring of exposure approach".

Chronic occupational exposure to inorganic mercury (mainly mercury vapor) may result in renal alterations affecting both tubuli and glomeruli. Most of the structural and/or functional renal changes become significant when urinary mercury (HgU) exceeds 50µg Hg/g creatinine. However, a

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marked reduction of the urinary excretion of prostaglandin E_2 was found at a HgU of 35µg Hg/g creatinine. Renal changes in moderately exposed workers are usually not related to the duration of mercury vapor exposure and the changes are reversible and mainly the consequence of recently absorbed mercury (last six months). Thus, monitoring HgU is useful for controlling the nephrotoxic risk of overexposure to inorganic mercury; HgU should remain below 50µg Hg/g creatinine in order to prevent cytotoxic and functional renal effects.

Several studies in lead-exposed workers with blood lead concentrations (PbB) usually below 70µg Pb/dL have disclosed either no renal effects or subclinical changes of marginal or unknown health significance. Changes in urinary excretion of eicosanoids was not found associated with deleterious consequences on the glomerular filtration rate (GFR, estimated as creatinine clearance rate) and renal hemodynamics if the workers' PbB was kept below 70µg Pb/dL during the whole professional career. The health significance of a slight renal hyperfiltration state in lead workers is yet unknown. In terms of lead body burden, a mean tibia lead concentration of about 60µg Pb/g bone mineral (that is 5 to 10 times the average "normal" concentration) and which corresponds to a cumulative PbB index of about 900µg Pb/dL × year is not affecting the GFR in male workers.

The cadmium concentration in urine (CdU) has been proposed as an indirect biological indicator for cadmium accumulation in the kidney cortex. Several biomarkers for detecting nephrotoxic effects of cadmium at different renal sites were studied in relation to CdU. In occupationally exposed male workers, there are distinct CdU thresholds for significant alterations of different renal markers ranging from 2.4 to 11.5µg Cd/g creatinine. A threshold of 10µg Cd/g creatinine (corresponding to 200µg Cd/g renal cortex: the critical Cd concentration in the kidney) is confirmed for the occurrence of low-molecular-mass proteinuria (functional effect) and subsequent loss of renal filtration reserve capacity. In workers, microproteinuria may be reversible when reduction or cessation of exposure occurs at a stage when tubular damage is still mild (β_2-microglobulinuria <1500µg/g creatinine) and CdU had never exceeded 20µg Cd/g creatinine. As the predictive significance of other renal changes (biochemical or cytotoxic) is still unknown, it seems prudent to recommend that the occupational exposure to cadmium should not allow CdU to exceed 5µg Cd/g creatinine.

These conclusions may not be extrapolated plainly to the general population, as recent studies have disclosed that some population subgroups are more vulnerable to exposure to these heavy metals than healthy male workers in the age range from 20 to 60 years.

Keywords

Health surveillance, industry, renal effects, cadmium, mercury, lead.

Introduction

Chronic occupational exposure to inorganic cadmium (Cd), lead (Pb), or mercury (Hg) may lead to nephropathy which usually starts insidiously and may evolve as a cascade of events leading from initial dysfunction and focal damage to a clinically detectable disease (1). As the kidney is known for its ability to compensate for renal damage, classical tests (e.g. serum creatinine or inulin clearance) are insensitive, since they only deviate late in the cascade of damage events when already a large part of the nephron mass is lost (2). Therefore, tools for detecting heavy metal nephrotoxicity at an early stage should be sensitive enough to detect early changes which ideally should be subclinical and reversible. Biomarkers of early renal effects are not diagnostic tests, but reflect that changes in renal integrity are occurring which may lead to clinical disease if exposure is not reduced. Knowledge about the predictive value of these biomarkers is particularly useful for preventive purposes.

To control the nephrotoxic risk of chronic exposure to cumulative metals such as cadmium, lead and mercury, an epidemiological approach can be used to define acceptable exposure levels. Therefore relationships between the dose of these metals and the probability of occurrence of adverse effects in populations at risk are necessary. Biomonitoring of the cumulative internal dose of these metals is possible through direct *in vivo* measurements using neutron activation analysis (NAA) or X-ray fluorescence (XRF) methods, such as for cadmium in kidney and liver (3), lead in bone (4), and mercury in kidney (5). However, these medical-physics techniques are not well suited for routine biomonitoring of exposure in the occupational settings. Therefore, indirect estimators are often used as surrogate biomarkers of the cumulative internal or target dose, *viz.* cadmium in urine (CdU), cumulative blood lead index (CBLI), and mercury in urine (HgU). These surrogate biomarkers of cumulative exposure have been found to correlate satisfactorily with kidney cortex cadmium in workers free of cadmium-induced proteinuria (3), tibial lead (4), and renal mercury (5), respectively. On the other hand, currently available analytical methods allow to determine small amounts of endogenous substances in urine and blood which may be useful as biomarkers of nephrotoxicity to assess function and integrity of specific nephron segments (6, 7).

We conducted several studies in groups of workers exposed to cadmium, lead or mercury vapor which allowed us to establish dose-effect or dose-response relations useful to derive surveillance strategies for the biomonitoring of prolonged exposure to these metals. The present paper gives an overview of our most significant findings of these epidemiological investigations.

Major epidemiological findings

Mercury

Excessive exposure to inorganic mercury compounds either through inhalation of elemental mercury vapor, ingestion of divalent mercury salts, or the use of skin-lightening cosmetics containing inorganic mercury may entail a nephrotic syndrome (severe albuminuria) or an acute tubular necrosis (8). It was previously believed that renal effects of exposure to mercury vapor occurred only at doses higher than those associated with the onset of signs and symptoms of the central nervous system, but numerous investigations during the last two decades have shown renal effects in workers at relatively low exposure levels (8). In several of these studies significant associations between biomarkers of renal effects and HgU were found which allowed to assess an HgU threshold value.

In 1980, we compared a group of 63 mercury-exposed workers (mean age 36) with an age-matched control group of 88 male subjects (9). The mean duration of exposure was 7 years and HgU averaged 59 µg Hg/g creatinine (range 5-200). An increased urinary excretion of high-molecular-mass proteins (albumin, transferrin, IgG) was indicative of an early glomerular dysfunction. The proximal tubular cells were also affected as shown by an increased urinary excretion of lysosomal β-galactosidase activity (BGALU). These biomarkers of glomerular and tubular effects correlated significantly with HgU. The likelihood of these nephrotoxic effects to be associated with exposure to mercury vapor increased significantly in workers with HgU >50 µg Hg/g creatinine (9). Another study, carried out five years later in a group of 180 male and female workers evidenced only proximal tubular effects, *viz*. increases in BGALU and urinary retinol-binding protein (RBPU). Again, the prevalence of abnormal values of these tubular markers rose significantly when HgU exceeded 50 µg Hg/g creatinine (10). In 1993, in the frame of a European network study, we assessed the usefulness of a battery of about 25 renal biomarkers in a cohort of 44 moderately exposed chloralkali workers (11). The mean duration of exposure was 11 years and HgU averaged 22 µg Hg/g creatinine (range 5-90)

which was less than half that in the two previous cohorts. Dose-response relations showed no changes in functional biomarkers since glomerular or tubular proteinuria was not detected. The main renal changes were indicative of tubular cytotoxicity, *viz.* increased leakage of tubular antigens and enzymes such as the brush-border lysosomal *N*-acetyl-*β*-*D*-glucosaminidase (NAG) and the S3-segment-specific intestinal alkaline phosphatase. Significant changes in biochemical indicators were also found, *viz*. decreases in urinary eicosanoids (PGE_2, $PGF_{2\alpha}$, TXB_2), glycosaminoglycans, and kallikrein. Most of these renal effects were found in workers excreting more than 50 µg Hg/g creatinine corroborating thus the adequacy of our previous proposal of this value as a HgU threshold (9, 10). However, a significant reduction in the urinary excretion of eicosanoids, especially PGE_2, was noted at HgU values as low as 35 µg Hg/g creatinine. None of the changes in this less exposed group were related to duration of exposure, which supports the view that renal changes induced by moderate exposure to mercury vapor are reversible and mainly the consequence of recently absorbed inorganic mercury.

It may be concluded from these three studies that, except a few biochemical changes such as a decrease in urinary pH and a reduction in PGE_2 excretion of which the health significance is not yet well understood, the nephrotoxic response to moderate occupational exposure to mercury vapor does not involve cytotoxic and functional alterations when HgU remains below 50 µg Hg/g creatinine. There is, however, an increased probability of cytotoxic effects at the proximal tubuli (e.g. enzymuria and increase in urinary tubular antigens) and functional changes (e.g. glomerular and tubular proteinuria, increase in serum β_2-microglobulin) when mercury vapor exposure chronically entails HgU concentrations above 50 µg Hg/g creatinine. Disrupted renal handling of low and high-molecular-mass proteins did not lead to clinically significant alterations of the kidney function, as glomerular and/or tubular proteinuria were shown to be reversible 9 to 12 months after cessation of exposure (10, 12). The time course of the reversibility seems to parallel the elimination kinetics of renal mercury. In a chloralkali worker with an HgU of 200 µg Hg/g creatinine, for example, the concentration of mercury in urine will slowly decrease to reach “acceptable” values (10-20 µg Hg/g creatinine) in about one year after removal from mercury vapor exposure, because the elimination half-time of HgU is on average about 90 days (13).

Lead

Chronic massive exposure to lead may cause progressive tubulo-interstitial nephropathy that develops insidiously and often leads to kidney fail-

ure (14-17). The level of lead exposure which may be associated with early adverse renal effects is however uncertain because incipient lead nephropathy is difficult to detect due to the lack of appropriate blood-borne or urinary biomarkers reflecting an early effect on the renal interstitial tissue. In chronically exposed lead workers, a decline of glomerular filtration rate (GFR) has been reported in cases with longstanding exposure and lead in blood (PbB) exceeding 70 µg Pb/dL (15, 16). A reduced GFR, however, indicates that lead nephropathy has already reached an irreversible stage. Hence, to better define the occupational exposure level without early adverse renal effects, a large number of cross-sectional studies were conducted during the last two decades in lead workers whose duration of exposure was less than 30 years and whose PbB usually remained below 60 µg Pb/dL (for a review, ref. 18). The majority of these studies and also the aforementioned European network study, involving the renal biomarker battery, disclosed a lack of association between the urinary excretion of renal markers of tubular or glomerular toxicity and biomarkers of lead exposure, *viz*. PbB, zinc-protoporphyrin in blood (ZPP), or tibia lead (9, 18-22). Some studies have revealed significant changes in the urinary excretion of the distal tubular enzyme kallikrein (23, 24), NAG (25-28), or eicosanoids, *viz*. two vasodilators 6-keto-$PGF_{1\alpha}$ and PGE_2 which decreased and a vasoconstrictor thromboxane B_2 which increased (22). The clinical significance of all these changes is not yet clear.

The crucial question is: What is the predictive value of these renal changes and should they be taken into account to assess the no-adverse-effect level of lead on the kidney?

First, the specificity of the reduced urinary activity of kallikrein (23, 24) and the increased excretion of NAG in urine (25-28) found in these studies can be questioned, because concomitant slight exposure to cadmium, which frequently occurs in lead industries, has not been considered as a possible confounder. Indeed, contrast analysis in groups of cadmium or lead workers showed CdU but not PbB as determinant of urinary kallikrein activity (29), and as to urinary NAG activity, its association with biomarkers of lead exposure (*viz*. PbB, ZPP, or tibia lead) was abolished after adjustment for CdU in stepwise multiple regression analysis (19, 30).

The changes in urinary excretion of eicosanoids might, however, reflect a disturbance of their synthesis in the kidney (22). As the renal production of eicosanoids may play a role in the regulation of the GFR, we advanced the hypothesis of a possible effect of lead on renal hemodynamics *before* other renal biomarkers get disturbed. To assess whether these eicosanoid changes had any impact on the renal hemodynamic response to an acute

oral protein load, we compared 76 male workers (mean age 44, range 29-56) moderately exposed to lead with 68 male controls matched for age and socio-economic status (19). The lead workers were exposed on average for 18 years (range 6-36), their historical PbBs rarely exceeded 70 µg Pb/dL, and they showed a threefold higher body burden of lead than the controls as estimated by *in vivo* XRF measurements of lead in tibia (66 *vs* 21 µg Pb/g bone mineral). The baseline and peak creatinine clearance rates averaged 116 and 132 mL/min in the control group against 121 and 141 mL/min in the lead-exposed group, respectively. All the subjects had a normal baseline creatinine clearance rate. In stepwise multiple regression analysis, both baseline and peak creatinine clearance rates were inversely associated with age ($p < 0.01$), whereas a modest but positive association emerged only with tibial bone lead ($p < 0.05$). These findings suggest that the cumulative lead exposure is associated with a slight state of renal hyperfiltration. In conclusion, it seems thus unlikely that the underlying changes in urinary eicosanoids have deleterious consequences on renal hemodynamics in this group of moderately exposed lead workers.

This study suggested that a mean tibia lead concentration of about 60 µg Pb/g bone mineral (that is about 5 to 10 times the average "normal" concentration) is not likely to affect the GFR and supports the view that adverse renal changes are unlikely to occur in adult male workers when the individual PbB is kept below 70 µg Pb/dL during the whole occupational career (31). However, in view of other biological changes at much lower PbBs, e.g. hematological and neurological effects, exposure to lead should not entail PbB values regularly exceeding 30 µg Pb/dL (32).

Cadmium

In humans, cadmium is a very cumulative toxic metal that under conditions of chronic exposure has the kidney as its critical target organ. Epidemiological studies performed in the 1970s and 1980s on groups of male cadmium workers have shown that cadmium may interfere with the renal handling of plasma-derived proteins usually characterized by microproteinuria due to impairment of tubular reabsorption of low-molecular-mass proteins, e.g. β_2-microglobulin (BMG) and RBP (33, 34). An isolated glomerular effect with increased permeability of high-molecular-mass proteins, e.g. albumin and transferrin, was less commonly found (34, 35). In male cadmium workers, the risk of microproteinuria increased significantly when CdU regularly exceeded 10 µg Cd/g creatinine which corresponds to a critical cadmium concentration in renal cortex of about 200 µg Cd/g wet weight as estimated by *in vivo* neutron activation analysis (3, 33, 36).

In the frame of the aforementioned collaborative network-project, involving laboratories of five European countries, we applied the battery of 25 renal tests on a cohort of 37 male cadmium workers (CdU: 2 to 16 µg Cd/g creatinine) and an age-matched control group (n = 43; CdU < 2 µg Cd/g creatinine). The aim was to assess the usefulness of various urinary and blood-borne analytes as potential biomarkers of cadmium toxicity on distinct nephron segments (37). The battery comprised *functional markers* (e.g. creatinine and BMG in serum; low and high-molecular-mass proteins in urine, and urinary calcium), *cytotoxicity markers* (e.g. tubular antigens or enzymes in urine), and *biochemical markers* (e.g. glycosaminoglycans, kallikein, sialic acids, and eicosanoids in urine). In this study, a broad spectrum of site-specific effects on the kidney with significant alterations in various markers of nephrotoxicity was found. Dose-response relations and logistic regressions between the renal markers and CdU showed that the probability of abnormal values significantly increased with CdU in a dose-dependent manner. Three main groups of CdU threshold values could be identified on the basis of various urinary biomarkers: one around *2µg Cd/g creatinine* associated with biochemical alterations (6-keto-$PGF_{1\alpha}$ and sialic acids), a second around *4µg Cd/g creatinine* associated with cytotoxic effects (renal brush-border antigens and NAG) and glomerular barrier dysfunction (albumine and transferrin), and a third around *10µg Cd/g creatinine* associated with dysfunction of tubular reabsorption (microproteinuria: BMG and RBP), hypercalciuria, and changes in other biomarkers (glycosaminoglycans).

Is there enough information as to the predictive value of these renal biomarkers to propose a meaningful biological exposure limit value for CdU?

Earlier retrospective findings showed that severe microproteinuria was irreversible in retired cadmium workers and was associated with raising serum creatinine (38) that in some workers turned into end-stage renal insufficiency (unpublished data). We have carried out three studies in order to better assess the health significance of cadmium-induced microproteinuria in male cadmium workers.

A 5-year prospective study was conducted in 23 cadmium workers removed from exposure because of the discovery of microproteinuria (39). They were exposed to cadmium for 25 years on average and at the time of the first examination the mean age of the group was 59 (46-68 years), the mean (SEM) CdU amounted to 22.2 (2.9) µg Cd/L, the geometric mean of urinary BMG (BMGU) and RBP (RBPU) amounted to 1770 and 1570 µg/L, respectively. Serum creatinine was normal (< 1.4 mg/dL) in all subjects, except in two (2.0 and 2.2 mg/dL). At the end of the study, BMGU

and RBPU were increased by about 50 and 30%, respectively. Moreover, serum creatinine significantly increased with time indicating a progressive reduction of the GFR, which was found to decrease five times more rapidly than what could be expected due to ageing alone. Elevated microproteinuria predicts thus an exacerbation of the age-related decline of the GFR, and hence, it should be regarded as an early adverse health effect.

The previous finding raised the question whether a CdU threshold value of 10 µg Cd/g creatinine prevents not only the occurrence of microproteinuria, but also the loss of nephron mass. In other words, an increased CdU, insufficient to modify the urinary excretion of plasma-derived microproteins, does it impair the renal filtration reserve capacity? Therefore, we have estimated the GFR in cadmium workers without (n = 31) or with (n = 12) increased microproteinuria, *viz.* BMGU and/or RBPU >300 µg/g creatinine (40). The subjects in both groups aged 50 to 64 (mean 55 years), had normal serum creatinine (<1.4 mg/dL), and the geometric mean (range) of CdU was 4.7 (2.1-8.8) and 11.1 (5.8-21.7) µg Cd/g creatinine, respectively. GFR was estimated as the creatinine clearance rate under baseline conditions and after an acute oral protein load to assess the hyperfiltration capacity of the kidney. The baseline creatinine clearance was normal in both groups (mean 116 mL/min). The creatinine clearance after protein load, however, failed to rise in the group with microproteinuria (mean 114 mL/min) and remained significantly lower than that in the group without microproteinuria (mean 124 mL/min). The mean peak creatinine clearance of the latter group was similar to that of an age-matched control group (n = 35; CdU <2 µg Cd/g creatinine). In conclusion, this study showed that the filtration reserve capacity of the kidney is lost when elevated microproteinuria is present, but there was no functional impairment at a renal cadmium burden that had not yet caused microproteinuria. This study validated thus the CdU estimate of 10 µg Cd/g creatinine as biological exposure limit to prevent the occurrence of microproteinuria in male cadmium workers.

The third study aimed at a more precise evaluation of the time trend of cadmium-induced microproteinuria by assessing its evolution in 32 cadmium workers according to criteria of CdU (cutoff 10 µg Cd/g creatinine) and severity of the microproteinuria at the time the exposure was substantially reduced or had ceased (41). The finding that 15 workers (63%) with CdU >10 µg Cd/g creatinine showed a BMGU exceeding the upper reference limit of 300 µg/g creatinine corroborated our earlier finding that the risk of abnormal microproteinuria dramatically increased when CdU regularly exceeded 10 µg Cd/g creatinine. When reduction of cadmium exposure took place and BMGU did not exceed 300 µg/g creatinine, the

risk of developing tubular dysfunction at a later stage was low even in cases with historical CdU values occasionally > 10 but always < 20 µg Cd/g creatinine. There is indication that the tubulotoxic effect of cadmium is reversible, provided that the historical CdU values had never exceeded 20 µg Cd/g creatinine and BMGU was mild (< 1,500 µg/g creatinine) at the time the cadmium exposure was reduced. When severe BMGU (> 1,500 µg/g creatinine) was found in combination with historical CdU values exceeding 20 µg Cd/g creatinine, cadmium-induced tubular dysfunction was progressive in spite of reduction or cessation of cadmium exposure.

These three studies on the health significance of microproteinuria confirmed thus the estimate of 10 µg Cd/g creatinine as the biological limit value of CdU above which the probability is high that cadmium-induced microproteinuria may develop. Hence, tubular proteinuria should be considered as an adverse health effect of cadmium exposure, because it can lead to irreversible renal damage associated with an exacerbation of the age-related decline in GFR and a decrease in the filtration reserve capacity. Since the health significance of renal changes other than microproteinuria is still unknown and no treatment is presently available to remove cadmium from its storage sites in the human organism, the adoption by the ACGIH of 5 µg Cd/g creatinine as biological exposure index (BEI) for cadmium seems justified (32). However, studies on the predictive value of renal changes other than microproteinuria are urgently needed to assess the validity of this BEI.

Conclusions

The results of the various epidemiological investigations performed since the 1970s by our laboratory indicate that in Belgium the efforts made by industries and the health authorities to reduce occupational exposure to inorganic mercury, lead, and cadmium are fully justified. Table 1 summarizes the guidelines for a practical and sound biological monitoring of prolonged exposure to these heavy metals in order to control for adverse nephrotoxic effects at an early stage and to ensure an adequate prevention strategy in industry. There are however sufficient findings now that the evidence from the occupational setting obtained in healthy male workers in the age range 20-60 years cannot be plainly extrapolated to the general population which includes much more vulnerable groups, such as children, women, and the elderly.

TABLE 1
Biological exposure thresholds for controlling health-significant nephrotoxic effects in adult male workers chronically exposed to moderate occupational levels of inorganic mercury, lead, or cadmium.

	Occupational exposure (healthy men; age 20-60)
Mercury (Hg)	**Hg in urine: 50 µg Hg/g creatinine** *Reversible effect: glomerular and/or tubular proteinuria*
Lead (Pb)	**Pb in blood: 70 µg Pb/dL** (31) *Decrease in GFR !?* *No validated early effect biomarker*
Cadmium (Cd)	**Cd in urine: 10 µg Cd/g creatinine** *Critical adverse health effect: microproteinuria* 5 µg Cd/g creatinine: BEI adopted by ACGIH (32)

GFR: glomerular filtration rate.
BEI: biological exposure index.
ACGIH: American Conference of Governmental Industrial Hygienists.

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