Markers of early renal changes induced by industrial pollutants. III Application to workers exposed to cadmium

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Abstract

Cadmium (Cd) was the third heavy metal investigated in the European collaborative research project on the development and validation of new markers of nephrotoxicity. Fifty workers exposed to Cd and 50 control workers were examined. After application of selection criteria 37 workers (mean age 43) exposed to Cd for an average of 11·3 years; and 43 age matched referents were retained for final analysis. The average concentrations of Cd in blood (Cd-B) and urine (Cd-U) of exposed workers were 5·5 μg Cd/l and 5·4 μg Cd/g creatinine respectively. By contrast with lead and mercury, Cd had a broad spectrum of effects on the kidney, producing significant alterations in amounts of almost all potential indicators of nephrotoxicity that were measured in urine—namely, low and high molecular weight proteins, kidney derived antigens or enzymes, prostanoids, and various other biochemical indices such as glycosaminoglycans and sialic acid. An increase in β_2-microglobulin and a decrease of sialic acid concentration were found in serum. Dose-effect/response relations could be established between most of these markers and Cd-U or Cd-B. The thresholds of Cd-U associated with a significantly higher probability of change in these indicators were estimated by logistic regression analysis. Three main groups of thresholds could be identified: one around 2 μg Cd/g creatinine mainly associated with biochemical alterations, a second around 4 μg Cd/g creatinine for high molecular weight proteins and some tubular antigens or enzymes, and a third one around 10 μg Cd/g creatinine for low molecular weight proteins and other indicators. The recent recommendation by the American Conference of Governmental Industrial Hygienists (ACGIH) of 5 μg Cd/g creatinine in urine as the biological exposure limit for occupational exposure to Cd appears thus justified, although for most of the effects occurring around this threshold the link with the subsequent development of overt Cd nephropathy is not established. In that respect, the very early interference with production of some prostanoids (threshold 2 μg Cd/g creatinine) deserves further investigation; although this effect might contribute to protect the filtration capacity of the kidneys, it might also play a part in the toxicity of Cd on bone.

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The kidney is the critical organ after long term occupational or environmental exposure to cadmium (Cd). Since the first description by Friberg,[1] of chronic Cd nephropathy, numerous epidemiological studies on industrial workers or on inhabitants of Cd polluted areas have documented the constellation of renal effects that may be produced by this heavy metal. As reviewed elsewhere[2–4] the nephrotoxic action of Cd can be detected on the basis of various urinary (low and high molecular weight proteins, enzymes, tubular antigens) or bloodborne (serum β_2-microglobulin (β_2-m) or creatinine) markers. Several

of these markers have been used to assess the concentration of Cd in kidney or urine associated with a risk of renal dysfunction. The critical concentrations vary with the selected renal effect. For instance the concentrations of Cd in urine (Cd-U) associated with a 10% risk of increased enzymuria or microproteinuria vary from about 5 to 10 μg/g creatinine.[2-5] The average concentrations of Cd in the kidney cortex corresponding to these values are 100–200 ppm.[6-10]

Cadmium is also presently the only industrial nephrotoxic chemical for which early indicators of toxicity have been validated. Follow up studies in workers exposed to Cd have clearly shown that the appearance of a persistent microproteinuria is the forerunner of a progressive deterioration of renal function.[11 12]

The selection of Cd in the framework of the European Community research project on the development and validation of new indicators of nephrotoxicity was of particular interest from more than one point of view. Firstly, because Cd is a well established nephrotoxin, affecting most of the markers examined in this project, the study presented a unique opportunity to compare the relative sensitivity of markers that have rarely been analysed in combination. Secondly, the application of a comprehensive battery of sensitive markers of nephrotoxicity may help to resolve the current controversy as to whether the critical concentration of Cd-U is closer to 10 μg/g creatinine as suggested by earlier studies mainly based on increased urinary excretion of low molecular weight proteins[13-15] or to 5 μg/g creatinine as proposed by more recent studies.[16-18] Finally, the determination of urinary prostaglandin concentration might provide insight into mechanisms in the progression of Cd nephropathy.

Subjects and methods

STUDY POPULATION

The study was conducted on 100 male workers recruited from two primary zinc and cadmium smelters in Belgium. The cohort of exposed workers included 40 subjects who were currently (n = 35) or previously (n = 5) employed in the Cd refinery departments of these plants and 10 workers who had been occupied until the early 1970s at horizontal retort zinc furnaces for the reduction of zinc calcine with coal. All the Cd workers had a Cd-U higher than 2 μg/g creatinine and had been exposed to Cd for at least one year (range 1·1 to 36·4 years). The occupational exposure to Cd ceased on average 11 (range 1–22) and 17 (range 15–19) years ago for the five Cd refinery and the 10 zinc furnace workers respectively. The control workers were recruited from the same plant but in departments where occupational exposure to heavy metals (Cd, lead, mercury) did not occur.

As in the two previous studies[19 20] a careful selection of the workers was made to exclude subjects whose renal function might be altered by causes other than exposure to Cd. In particular, it was ascertained that all control or exposed workers had a blood lead concentration below 350 μg/l and a concentration of mercury in urine lower than 5 μg/g creatinine, and that control workers had a Cd-U below 2 μg/g creatinine. Also, the results obtained on urine sam-

Table 1 Characteristics of control and cadmium exposed workers

	Control workers (n = 43)		*Exposed workers (n = 37)*		
	Mean or No	*SD or (range)*	*Mean or No*	*SD or (range)*	*p Value**
Age (y)	43·2	10·8	44·9	10·0	NS
Body mass index	25·6	2·3	25·6	3·6	NS
Smokers	15		15		NS
Duration (y)	12·0	8·4	6·9	9·4	NS
Cigarettes/day	23·5	11·4	28·3	10·2	NS
Alcohol drinkers	33		25		NS
Glasses/week	10·8	7·6	8·2	6·1	NS
Exposure (y)	—		11·3	7·2	—
Cd-U† (μg/l)	1·11	(0·27–2·85)	6·72	(1·39–36·1)	<0·0001
Cd-U† (μg/g creatinine)	0·69	(0·10–1·90)	5·39	(2·10–16·40)	<0·001
Cd-B† (μg/l)	0·84	(0·30–2·8)	5·52	(1·6–14·6)	<0·0001
Hg in urine † (μg/l)	2·97	(1·50–9·90)	2·56	(1·50–7·40)	NS
Hg in urine† (μg/g creatinine)	1·86	(0·80–4·80)	2·04	(0·70–4·00)	NS
Pb in blood (μg/l)	126	55	154	65	NS
ZPP in blood (μg/g Hb)	1·19	0·63	1·25	0·33	NS
Creatinine (g/l)	1·72	0·64	1·37	0·57	<0·05

*Student's t test or χ^2 test.
†Geometric means.

Table 2 Significant associations found by multiple regression analysis

*Dependent variables**	*Independent variables**	*Partial regression coefficient*	*Partial r^2*	*p Value*
Urine:				
Albumin‡	Crt-U	0·837	0·228	0·0001
	Cd-U	0·211	0·081	0·0037
Transferrin†‡	Crt-U	0·869	0·168	0·0002
	Cd-U	0·265	0·084	0·0042
IgG‡	Crt-U	1·031	0·272	0·0001
	Drinker	−0·179	0·043	0·0306
β_2-m‡	Cd-U	0·280	0·109	0·0029
RBP‡	Crt-U	0·773	0·195	0·0001
	Drinker	−0·180	0·055	0·0195
Protein 1†	Crt-U	1·027	0·241	0·0001
	Smoker	−0·173	0·039	0·0443
THG	Crt-U	0·879	0·351	0·0001
	Cd-U	0·127	0·043	0·0220
NAG‡	Crt-U	0·642	0·283	0·0001
	Cd-U	0·164	0·103	0·0006
BB50†	Crt-U	0·618	0·145	0·0005
	Cd-U	0·150	0·048	0·0358
BBA	Cd-U	0·183	0·109	0·0028
	Crt-U	0·400	0·066	0·0151
HF5	Crt-U	0·392	0·093	0·0061
	Cd-U	0·141	0·063	0·0188
	Crt-U	0·670	0·098	0·0010
IAP‡	Cd-U	0·366	0·251	0·0001
	Body mass index	0·028	0·035	0·0410
TNAP	Crt-U	1·111	0·161	0·0002
Fibronectin	Crt-U	0·452	0·092	0·0062
	Smoker	0·126	0·049	0·0392
6-keto-PGF_{1_α}	Crt-U	0·787	0·333	0·0001
	Cd-B	0·175	0·122	0·0001
PGE_2	Crt-U	0·790	0·207	0·0001
	Cd-B	0·252	0·159	0·0001
PGF_{2_α}	Crt-U	1·307	0·385	0·0001
TXB_2	Crt-U	0·608	0·253	0·0001
Kallikrein	Crt-U	0·818	0·275	0·0001
GAG	Crt-U	0·648	0·707	0·0001
	Cd-U	0·040	0·023	0·0118
	Drinker	−0·043	0·015	0·0357
Sialic acid	Crt-U	1·007	0·760	0·0001
	Cd-B	0·095	0·040	0·0002
Blood:				
Creatinine/serum†	Smoker	−0·522	0·059	0·0294
β_2-m/serum‡	Cd-U	0·375	0·185	0·0001
Sialic acid/plasma†	Cd-B	−42·02	0·062	0·0261
Sialic acid/RBC	Cd-U	−1·276	0·054	0·0378

*All urinary variables (expressed per litre of urine) and Cd-B were log transformed.
†Standardised for age.
‡Associated to duration of exposure in the exposed group. For abbreviations see subjects and methods section.

ples with a creatinine concentration lower than 0·3 or higher than 4 g/l were discarded and for the assay of kallikrein, those obtained in subjects with a natriuria lower than 35 mmol/g creatinine were excluded as well. After that selection, 37 workers were retained in the Cd cohort (26 currently and 11 previously exposed) and 43 in the control cohort.

Methods

The collection of biological samples was according to the protocol in the two previous studies.[19 20] The battery of tests was almost identical, comprising, in blood: lead, cadmium (Cd-B) and zinc protoporphyrin (ZPP) concentrations, alcian blue (AB) binding to red blood cell (RBC) surfaces, sialic acid content of RBC membranes; in serum or plasma: creatinine (crt-S), β_2-m, sialic acid, antiglomerular basement membrane antibodies (anti-GBM); and in urine:Cd-U, mercury (Hg-U), sodium, creatinine (crt-U), pH, albumin, transferrin, immunoglobulin (IgG), β_2-m, retinol binding protein (RBP), protein

Table 3 Urinary and bloodborne markers of nephrotoxicity in controls and workers exposed to Cd

Marker	*Control workers (n = 43)§ Mean (SD or range)*	*Exposed workers (n = 37)| Mean (SD or range)*	*p Value**
Urine:†			
Albumin (mg/l)	8·3 (3·7–40·0)	11·1 (3·8–198)	NS
Transferrin (μg/l)	271 (68–2464)	471 (72–8429)	<0·01
IgG (μg/l)	1850 (381–11313)	1764 (499–9432)	NS
β_2-m (μg/l)	76 (9–440)	112 (19–5508)	NS
RBP (μg/l)	83 (27–608)	94 (34–2737)	NS
Protein 1 (μg/l)	123 (33–609)	134 (15–3566)	NS
THG (mg/l)	24·9 (6·0–66·4)	31·5 (9·8–160)	NS
NAG (U/l)	1·32 (0·20–2·70)	1·62 (0·70–5·91)	NS
BB50 (U/l)	4·2 (0·8–17·2)	6·1 (1·7–91·4)	<0·05
BBA (U/l)	3·7 (0·9–24·4)	5·3 (1·8–108·9)	<0·05
HF5 (U/l)	5·8 (2·4–17·8)	7·4 (1·7–133)	NS
IAP (U/l)	0·86 (0·19–4·9)	1·53 (0·26–13·1)	<0·01
TNAP (U/l)	0·272 (0·012–2·38)	0·403 (0·008–3·48)	NS
Fibronectin (μg/l)	15·3 (4·0–103)	15·0 (3·6–37·8)	NS
6-keto-$PGF_{1\alpha}$ (ng/l)	165 (37–322)	243 (75–438)	<0·00001
PGE_2 (ng/l)	216 (85–548)	329 (97–947)	<0·01
$PGF_{2\alpha}$ (ng/l)	337 (108–1803)	353 (31–1308)	NS
TXB_2 (ng/l)	66 (2–235)	68 (22–186)	NS
Kallikrein (U/l)	0·72 (0·20–3·17)	0·82 (0·22–1·86)	NS
GAG (mg/l)	45·2 (33·4–83·3)	47·9 (25·0–86·6)	NS
Sialic acid (mg/l)	355 (238–472)	425 (243–878)	<0·001
Blood:‡			
Creatinine/serum (mg/l)	10·3 (1·0)	9·8 (1·0)	<0·05
β_2-m/serum (mg/l)	1·39 (0·39)	1·75 (0·41)	<0·001
Sialic acid/plasma (mg/l)	629 (79)	600 (109)	NS
Sialic acid/RBC (μg/mg protein)	25·7 (2·7)	24·9 (2·7)	NS
AB binding/RBC (ng/10^6 RBC)	197 (15)	191 (19)	NS
Anti-GBM/serum (U/l)	25·4 (7·4)	23·9 (7·0)	NS

*Student's *t* test.
†Geometric means; ‡Arithmetic means.
§For PGE_2 and kallikrein n = 39.
|For PGE_2 n = 35.
For the abbreviations see subjects and methods section.
All the markers of nephrotoxicity were standardised for the determinants unrelated to Cd exposure (see table 2). Standardisation was based on the mean of the total population.

1, Tamm-Horsfall glycoprotein (THG), fibronectin, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane B_2 (TXB_2), sialic acid, glycosaminoglycans (GAG), the BBA, BB50, and HF5 brush border antigens, and the activities of kallikrein, intestinal alkaline phosphatase (IAP), tissue nonspecific alkaline phosphatase (TNAP), and N-acetyl-β-D-glucosaminidase (NAG).

Statistical analysis was performed as described earlier.[19]

Results

Table 1 outlines the general characteristics and exposure parameters of workers exposed to Cd and their controls. No significant differences were found between the two cohorts for age, body mass index, smoking and drinking habits, or exposure to lead or mercury. The mean crt-U was slightly lower in exposed workers. By contrast Cd-U or Cd-B were on the average six to eight times higher than those of the control group. The mean duration of exposure was 11·3 years.

In the control group, the urinary excretion of transferrin, protein 1, and BB50, and also crt-S (serum) and sialic acid concentration in plasma showed a positive association with age. Multivariate correlation analysis was performed on the whole population (n = 80) after standardisation of these five variables for age. Table 2 lists the determinants significantly associated with the dependent variables. When associations were found with both Cd-B and Cd-U (tested separately), however, only the strongest one is mentioned in this table. All urinary parameters, except β_2-m, were positively correlated with crt-U. Significant positive associations were found between Cd-U and concentrations of albumin, transferrin, β_2-m, NAG, GAG, and most tubular antigens (THG, BB50, BBA, HF5, and IAP).

Table 4 Prevalences of abnormal values of urinary and bloodborne markers of nephrotoxicity in controls and workers exposed to Cd

Marker	*Cut off value†*	*Control workers (n = 43)‡* No	(%)	*Exposed workers (n = 37)§* No	(%)	*p Value**
Urine:						
Albumin	>19·0	2	4·7	7	18·9	NS
Transferrin	>903	2	4·7	8	21·6	<0·05
IgG	>5042	2	4·7	4	10·8	NS
β_2-m	>279	2	4·7	5	13·5	NS
RBP	>184	2	4·7	3	8·1	NS
Protein 1	>340	2	4·7	4	10·8	NS
THG	>50·1	2	4·7	7	18·9	NS
NAG	>2·19	2	4·7	8	21·6	<0·05
BB50	>15·6	2	4·7	3	8·1	NS
BBA	>6·7	2	4·7	9	24·3	<0·05
HF5	>12·6	2	4·7	6	16·2	NS
IAP	>2·72	2	4·7	8	21·6	<0·05
TNAP	>1·137	2	4·7	7	18·9	NS
Fibronectin	>41·7	2	4·7	0	—	NS
6-keto-PGF_{1_2}	>269	2	4·7	18	48·6	<0·00001
PGE_2	>531	2	5·1	6	17·1	NS
PGF_{2_2}	>1094	2	4·7	1	2·7	NS
TXB_2	>146	2	4·7	1	2·7	NS
Kallikrein	<0·255	2	5·1	1	2·7	NS
GAG	>56·9	2	4·7	4	10·8	NS
Sialic acid	>467	2	4·7	13	35·1	<0·001
Blood:						
Creatinine/serum	>11·9	2	4·7	1	2·7	NS
β_2-m/serum	>2·16	2	4·7	6	16·2	NS
Sialic acid/plasma	<495	2	4·7	5	13·5	NS
Sialic acid/RBC	<22·5	1	2·3	6	16·2	NS
AB binding/RBC	<166	1	2·3	3	8·1	NS
Anti-GBM/serum	>38·7	2	4·7	2	5·4	NS

*Fisher's exact test.
†For units see table 3 and for abbreviations see subjects and methods section.
‡For PGE_2 and kallikrein n = 39.
§For PGE_2 n = 35.
All the markers of nephrotoxicity were standardised for the determinants unrelated to Cd exposure (see table 2). Standardisation was based on the mean of the total population.

A positive association was found between Cd-B and 6-keto-PGF_{1_2} and PGE_2 concentrations in urine. Concentration of sialic acid in urine was positively related to Cd-B whereas sialic acid concentration in plasma and in RBC membranes showed an inverse relation with Cd-B and Cd-U respectively. A positive association of Cd-U with β_2-m, but not creatinine concentrations, in serum was also found. Eight parameters were significantly correlated with the duration of exposure in the group exposed to Cd—namely, albumin, transferrin, IgG, β_2-m, RBP, NAG, and IAP concentrations in urine, and β_2-m in serum.

After standardisation for determinants unrelated to exposure to Cd (including crt-U), Cd workers showed on average a significantly higher urinary excretion of transferrin, BB50, BBA, IAP, 6-keto-PGF_{1_2}, PGE_2, and sialic acid. A significant increase in β_2-m in serum was also found whereas crt-S (serum) was slightly diminished (table 3). The comparison of prevalences of abnormal values between the two cohorts (table 4) showed a pattern that was consistent with that found for the mean values in table 3. Tables 5 and 6 and figs 1 and 2 show dose-effect and dose-response relations. They were established by using as landmarks the concentrations of 2 and 10 μg/l for Cd-B and 2 and 10 μg/g creatinine for Cd-U. Regarding Cd-U, which is mainly a reflection of the body burden of Cd, a significant increase in the mean values or the prevalences of abnormal values was found for the highest exposure group (Cd-U >10 μg/g creatinine) for all markers measured in urine, except IgG, TNAP, fibronectin, PGE_2, PGF_{2_2}, TXB_2, and kallikrein. For 6-keto-PGF_{1_2}, the increase was already significant in the group with Cd-U between 2 and 10 μg/g creatinine. The rise of PGE_2 in urine, by contrast, was not dose related as it reached statistical significance only in the intermediate exposure group. It should be noted, however, that two high values had to be discarded in the group with Cd-U above

Table 5 Mean values of urinary and bloodborne markers of nephrotoxicity as a function of Cd-U (μg/g creatinine)

Marker	*Cd-U < 2 (n = 43)*‡		*2 ≤ Cd-U ≤ 10 (n = 30)*‖		*Cd-U > 10 (n = 7)*§¶	
Urine:*						
Albumin	8·3	(a)**	9·1	(a)	26·9	(b)
Transferrin	271	(a)	385	(a)	1112	(b)
IgG	1850	(a)	1698	(a)	2080	(a)
β_2-m	76	(a)	103	(ab)	163	(b)
RBP	83	(a)	82	(a)	165	(b)
Protein 1	123	(a)	107	(a)	355	(b)
THG	24·9	(a)	30·4	(a)	36·9	(a)
NAG	1·32	(a)	1·45	(a)	2·59	(b)
BB50	4·2	(a)	5·8	(a)	7·4	(b)
BBA	3·7	(a)	5·0	(ab)	7·1	(b)
HF5	5·8	(a)	6·9	(ab)	10·2	(b)
IAP	0·86	(a)	1·24	(a)	3·83	(b)
TNAP	0·272	(a)	0·399	(a)	0·417	(a)
Fibronectin	15·3	(a)	14·8	(a)	16·2	(a)
6-keto-$PGF_{1\alpha}$	165	(a)	231	(b)	302	(b)
PGE_2	216	(a)	352	(b)	222	(ab)
$PGF_{2\alpha}$	337	(a)	369	(a)	293	(a)
TXB_2	66	(a)	64	(a)	84	(a)
Kallikrein	0·72	(a)	0·86	(a)	0·68	(a)
GAG	45·2	(a)	46·5	(a)	54·2	(b)
Sialic acid	355	(a)	406	(a)	517	(b)
Blood:†						
Creatinine/serum	10·3	(a)	9·8	(a)	9·7	(a)
β_2-m/serum	1·39	(a)	1·69	(b)	2·03	(c)
Sialic acid/plasma	629	(a)	599	(a)	608	(a)
Sialic acid/RBC	25·7	(a)	25·1	(a)	24·2	(a)
AB binding/RBC	197	(a)	190	(a)	195	(a)
Anti-GBM/serum	25·4	(a)	24·1	(a)	23·0	(a)

*Geometric means; †Arithmetic means.
‡For PGE_2 and kallikrein n = 39.
§For PGE_2 n = 5.
‖¶Average duration of Cd exposure was 9·7 and 17·9 years respectively.
**Means with the same letter do not differ significantly (Duncan's test).
For units see table 3 and for abbreviations see subjects and methods section.
All the markers of nephrotoxicity were standardised for the determinants unrelated to Cd exposure (see table 2). Standardisation was based on the mean of the total population.

10 μg/g creatinine because of suspected seminal contamination. Of the bloodborne parameters, only serum β_2-m concentrations showed changes related to Cd-U (see table 5 and fig 1).

As a general rule, the dose dependence of renal effects is less evident when the classification is based on Cd-B, which is not surprising as this parameter mainly reflects recent exposure to the metal. A noteworthy finding, however, is that both urinary PGE_2 and 6-keto-$PGF_{1\alpha}$ show significant dose-effect or response relations with Cd-B (see table 6 and fig 2).

A logistic regression model was applied to the exposed group to better delineate the dose-response relations between the renal variables and Cd-B, Cd-U, or duration of exposure. For Cd-U, however, because of the high sensitivity of some markers, this analysis was performed by combining the exposed group and the control workers excreting more than 1 μg Cd/g creatinine. The reference group was thus redefined as subjects with a Cd-U value lower than 1 μg/g creatinine. The 95th or the 5th percentile values of this group were used as upper or lower limits of normal for all parameters. Figure 3 shows the relations emerging with Cd-U, separately for plasma derived proteins, enzymes or antigens derived from the proximal tubule, and other indicators.

The most sensitive urinary markers were 6-keto-$PGF_{1\alpha}$ and sialic acid, followed by high molecular weight proteins (albumin and transferrin) together with some tubular antigens or enzymes (BBA, IAP, and NAG), then THG, and eventually low molecular weight proteins (β_2-m, RBP), HF5, and TNAP. In that sensitivity scale serum β_2-m appeared immediately after high molecular weight proteins.

The thresholds of Cd-U associated with a probability of change significantly higher than in the control group ranged from 2·3 (6-keto-$PGF_{1\alpha}$) to 11 (β_2-microglobulinuria) μg Cd/g creatinine. It is possible to distinguish three main groups of thresholds

Table 6 Mean values of urinary and bloodborne markers of nephrotoxicity as a function of Cd-B (μg/l)

Marker	*Cd-B < 2 (n = 42)*‡		*2 ⩽ Cd-B ⩽ 10 (n = 32)*‖		*Cd-B > 10 (n = 6)*§¶	
Urine:*						
Albumin	8·1	(a)**	10·8	(a)	14·5	(a)
Transferrin	265	(a)	454	(ab)	611	(b)
IgG	1799	(a)	2133	(a)	2350	(a)
β_2-m	73	(a)	104	(ab)	205	(b)
RBP	84	(a)	88	(a)	118	(a)
Protein 1	123	(a)	126	(a)	181	(a)
THG	25·2	(a)	30·8	(a)	31·2	(a)
NAG	1·30	(a)	1·55	(ab)	2·17	(b)
BB50	4·19	(a)	6·22	(a)	5·40	(a)
BBA	3·64	(a)	5·43	(a)	4·83	(a)
HF5	5·93	(a)	7·69	(a)	5·25	(a)
IAP	0·849	(a)	1·48	(ab)	1·84	(b)
TNAP	0·268	(ab)	0·476	(a)	0·170	(a)
Fibronectin	15·8	(a)	14·8	(a)	13·4	(a)
6-keto-$PGF_{1\alpha}$	166	(a)	231	(b)	280	(b)
PGE_2	218	(a)	308	(ab)	457	(b)
$PGF_{2\alpha}$	339	(a)	347	(a)	356	(a)
TXB_2	65	(a)	68	(a)	74	(a)
Kallikrein	0·76	(a)	0·76	(a)	0·89	(a)
GAG	44·8	(a)	47·5	(a)	53·0	(b)
Sialic acid	354	(a)	417	(ab)	473	(b)
Blood:†						
Creatinine/serum	10·2	(a)	10·1	(a)	9·8	(a)
β_2-m/serum	1·40	(a)	1·72	(ab)	1·79	(b)
Sialic acid/plasma	632	(a)	604	(ab)	568	(b)
Sialic acid/RBC	25·7	(a)	25·0	(a)	24·9	(a)
AB binding/RBC	194	(a)	192	(a)	202	(a)
Anti-GBM/serum	25·2	(a)	24·4	(a)	23·1	(a)

*Geometric means; †Arithmetic means.
‡For PGE_2 and kallikrein n = 38.
§For PGE_2 n = 4.
‖¶Average duration of exposure to Cd was 11·3 and 12·0 years respectively.
**Means with the same letter do not differ significantly (Duncan's test).
For units see table 3 and for abbreviations see subjects and methods section.
All the markers of nephrotoxicity were standardised for the determinants unrelated to Cd exposure (see table 2). Standardisation was based on the mean of the total population.

associated with the occurrence of renal changes: a threshold around 2 μg Cd/g creatinine for 6-keto-$PGF_{1\alpha}$ and sialic acid (2·4 μg Cd/g creatinine), around 4 μg Cd/g creatinine for BBA, NAG, IAP, albumin, and transferrin (3·7, 4·0, 4·1, 4·1, and 3·6 μg Cd/g creatinine respectively), and a threshold around 10 μg Cd/g creatinine for TNAP, HF5, RBP, β_2-m, and GAG (9·7, 10, 10·4, 11·5, and 11·5 μg Cd/g creatinine respectively). Serum β_2-m and urinary THG had intermediate thresholds of 6·1 and 7 μg Cd/g creatinine respectively.

When the logistic regression analysis was performed with Cd-B as the independent variable, relations similar to those shown in fig 3 were obtained with three main groups of thresholds around 2, 4, and 10 μg Cd/l. Three variables were also significantly related to the duration of exposure by logistic regression analysis—namely, albumin, transferrin, and IAP. For these parameters, the thresholds corresponding to a significant increase in the probability of change were 10·8, 8·7, and 8·3 years respectively.

Logistic regression analysis only on workers currently exposed to Cd (n = 26) and control subjects with a Cd-U higher than 1 μg/g creatinine produced thresholds that did not differ greatly from those mentioned, taking into account the uncertainty due to the lower number of subjects. Thresholds around 2 μg Cd/g creatinine and that for serum β_2-m were unchanged, those around 4 μg Cd/g creatinine were on average 10% lower, whereas those around 10 μg Cd/g creatinine were 20% higher. The only threshold that was clearly different was for THG (14 instead of 7 μg Cd/g creatinine).

Discussion

Workers examined in the present study were only moderately exposed to Cd as only a few (six or seven

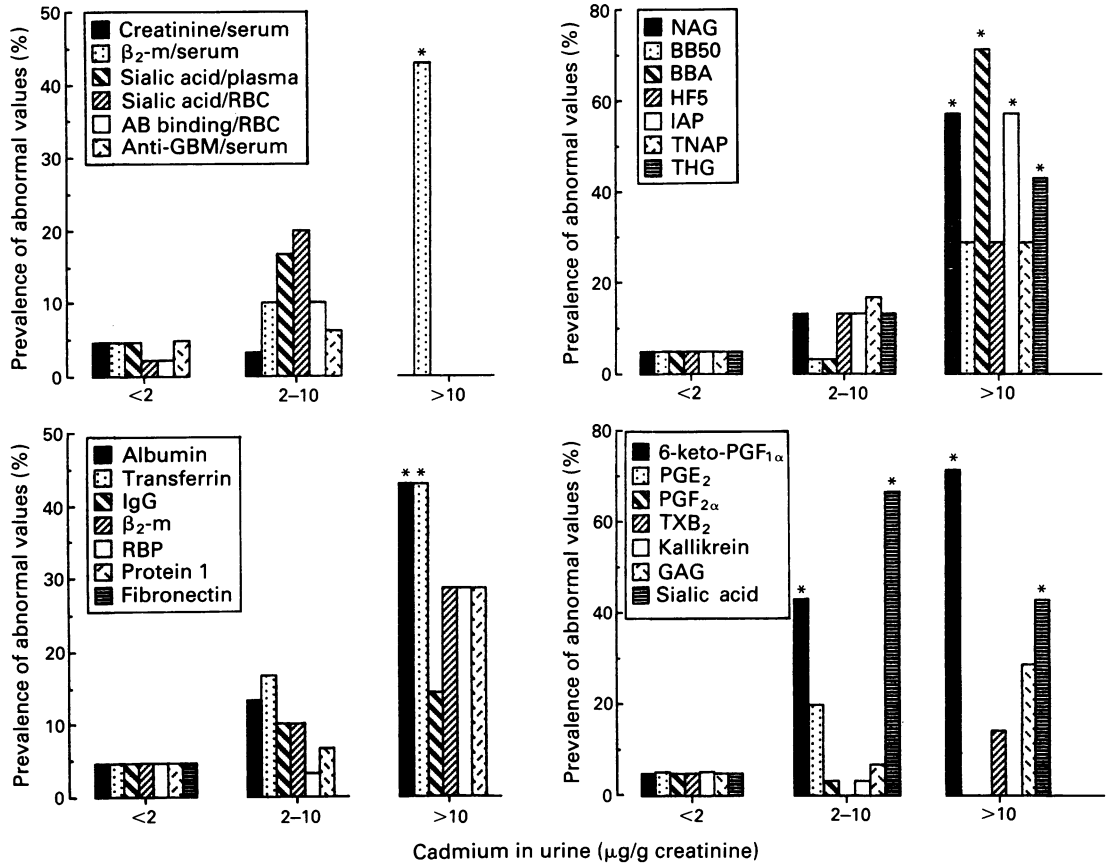

*Figure 1 Prevalences of abnormal values of markers of nephrotoxicity as a function of Cd-U in workers exposed to Cd and their referents. Prevalences were established on the basis of the cut off values given in table 4. For group size and duration of exposure, see table 5. *Significantly different from the group with Cd-U < 2 μg Cd/g creatinine.*

subjects) had Cd-B or Cd-U exceeding the previously proposed critical levels (10 μg Cd/l and 10 μg Cd/g creatinine).[13-15] Despite this moderate exposure, most potential indicators of nephrotoxicity showed alterations associated with the internal dose of Cd (table 2).

It is clear that these markers do not respond to exposure to Cd with the same sensitivity. Urinary 6-keto-$PGF_{1\alpha}$ seems to be the most sensitive marker with up to 49% of abnormally increased values in the Cd group. This marker is followed by urinary sialic acid (35% of raised values), tubular antigens or enzymes (BBA, NAG, IAP, around 20%), and then high or low molecular weight proteins. Logistic regression analysis allowed us to derive the thresholds of Cd-U from which the probability of detecting abnormalities in these markers rises significantly. The comparatively low number of subjects and the discrete alterations of some markers (for example, β_2-m and RBP) preclude a very precise estimate of some thresholds. Nevertheless, this logistic regression analysis supported by the dose-response relations showed the existence of three main groups of thresholds for Cd-U; one around 2 μg Cd/g creatinine essentially associated with biochemical changes (increased urinary 6-keto-$PGF_{1\alpha}$ and sialic acid concentrations), a second around 4 μg Cd/g creatinine for high molecular weight proteins and some tubular antigens or enzymes (BBA, NAG), and a third one around 10 μg Cd/g creatinine for low molecular weight proteins and other indicators; the last corresponding to the previously proposed biological threshold for Cd nephropathy.[6 13 15]

These data are thus in agreement with previous studies that have reported the occurrence of some renal effects in workers excreting less than 10 μg Cd/g creatinine. In those studies, as in the present one, the effects consisted mainly of an increased urinary

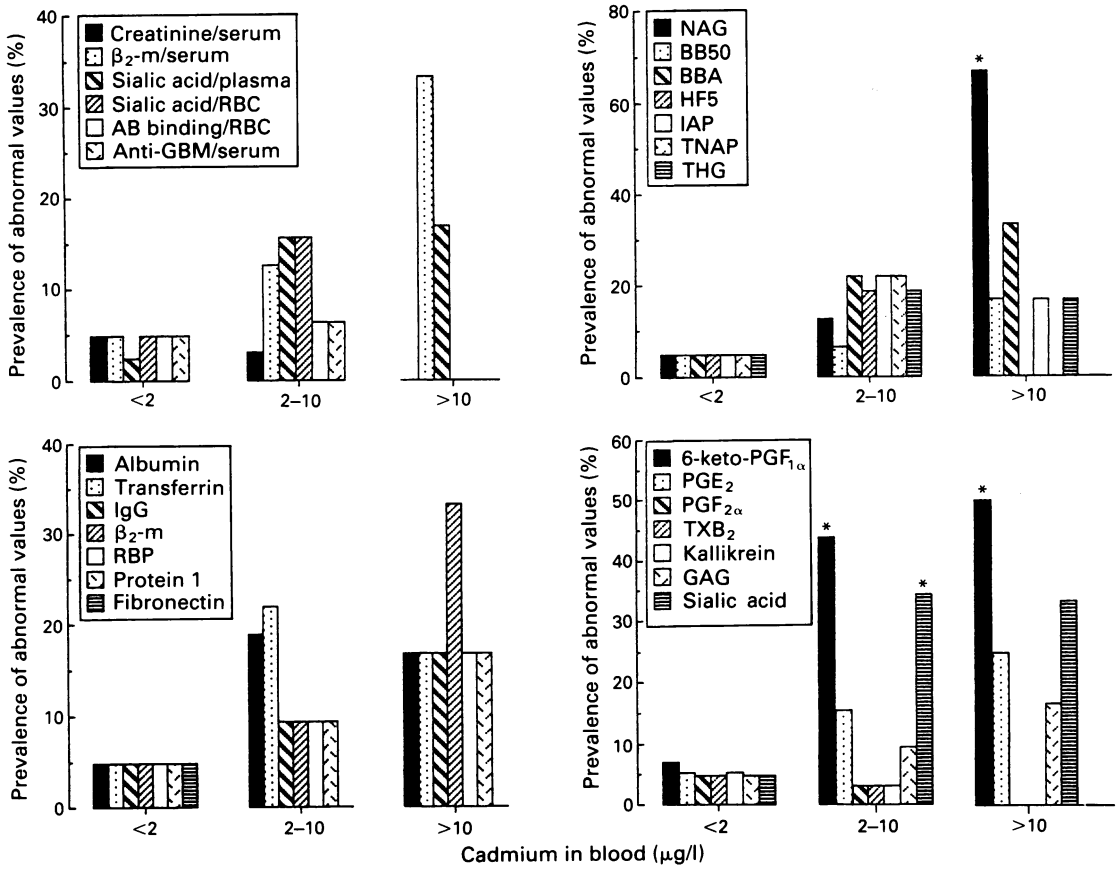

*Figure 2 Prevalences of abnormal values of markers of nephrotoxicity as a function of Cd-B in workers exposed to Cd and their referents. Prevalences were established on the basis of the cut off values given in table 4. For group size and duration of exposure, see table 6. *Significantly different from the group with Cd-B < 2 μg Cd/l.*

excretion of tubular enzymes or antigens, although some authors have also found an increase in β_2-microglobulinuria (for review see[5]). The thresholds of Cd-U derived in some of these studies are also in accordance with our estimates. For instance Mueller *et al*[16] found a 10% risk of raised NAG or alanine aminopeptidase activity in urine from Cd-U of 6·3 and 5 μg Cd/g creatinine. Verschoor *et al*[17] also found that urinary NAG was significantly increased in workers with Cd-U above 5 μg Cd/g creatinine. A somewhat lower threshold of Cd-U (3 μg Cd/g creatinine) was reported for NAG by Chia *et al*[21] but this was based on a study of female workers. Using a two phase linear regression model to estimate a threshold of cumulative exposure to Cd in air for the occurrence of renal changes, Mason *et al*[9] also noted that the inflection point of urinary NAG activity was at substantially lower Cd concentrations in air than that of other variables (low or high molecular weight proteins), which is in agreement with our findings for Cd-U. The question that now arises is which of these thresholds must be adopted as the maximum permissible value for occupational exposure to Cd? The answer to that question depends on the health significance of the renal effects; that is, whether they are predictive or not of a progressive decline in renal function. For low molecular weight proteins, for which the threshold seems to be definitively around 10 μg Cd/g creatinine, it is well established that their increased urinary excretion is irreversible and may lead to an exacerbation of the age related decline in the glomerular filtration rate.[11,12] More recently, we have shown that a Cd body burden insufficient to cause microproteinuria (Cd-U below 10 μg Cd/g creatinine) does not compromise the filtration reserve capacity of the kidney, and this finding was interpreted as evidence that the threshold of 10 μg Cd/g creatinine affords an adequate protection.[22] Renal

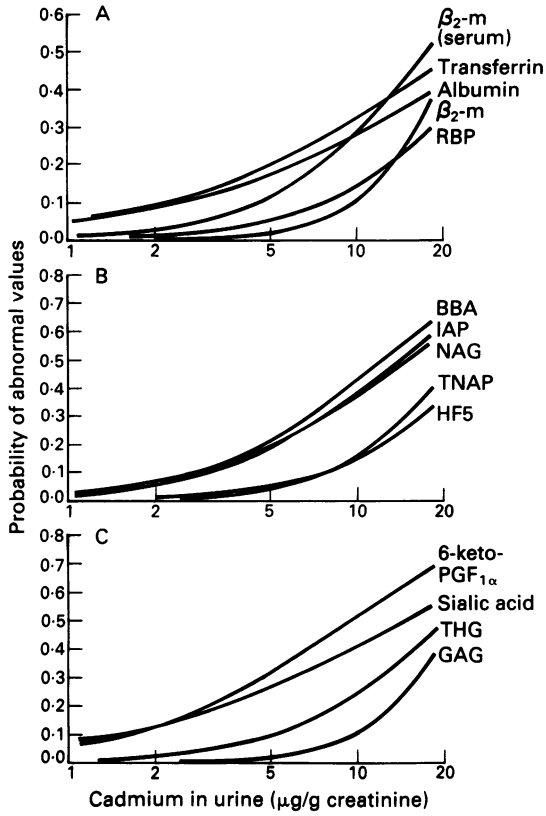

Figure 3 Probability of renal effects as a function of Cd-U for (A) urinary proteins and serum β_2-m, (B) enzymes or antigens in urine derived from the proximal tubule, and (C) other urinary markers. The upper limits of normal, defined as the 95th percentile of the values in control subjects with Cd-U lower than 1 μg Cd/g creatinine were as follows (see table 3 for units): albumin, 19; transferrin, 903; β_2-m, 324 (in serum: 2·17); RBP, 190; BBA, 6·7; IAP, 2·72; NAG, 2·19; TNAP, 1·9; HF5, 12·6; 6-keto-PGF$_{1\alpha}$, 280; sialic acid, 501; THG, 50·1; and GAG, 63·2. The p values are: albumin 0·114, transferrin 0·078, β_2-m 0·099 (in serum 0·029), RBP 0·12, BBA 0·016, IAP 0·021, NAG 0·028, TNAP 0·068, HF5 0·094, 6-keto-PGF$_{1\alpha}$ 0·016, sialic acid 0·052, THG 0·043, and GAG 0·099. All the markers of nephrotoxicity were standardised for the determinants unrelated to Cd exposure (see table 2). Standardisation was based on the mean of the total population.

effects that occurred from a threshold of Cd-U lower than 10 μg Cd/g creatinine consisted mostly of an increased leakage of tubular antigens or enzymes into urine. These changes, like microproteins, were related to Cd-U as well as to Cd-B, which indicates that they are not necessarily reversible effects due to the recently absorbed Cd. The enhanced excretion of tubular enzymes or antigens in the urine of workers exposed to Cd might be the consequence of either an exfoliation of damaged tubular cells or an increased turnover of tubular cells or some of their constituents (for example, brush border membrane antigens), or else some metabolic disturbances. Depending on the underlying mechanism some of these changes might be completely reversible and not necessarily an early sign of an irreversible tubular dysfunction.

These early tubular changes were accompanied by an increased urinary excretion of albumin and transferrin. Because in this range of Cd body burden the protein reabsorption capacity of tubular cells is not yet impaired, the glomerular type proteinuria may be ascribed to a loss of glomerular barrier function, resulting probably, as discussed elsewhere,[23 24] from a depletion of the glomerular polyanion charge. This suggests that subtle alterations of the glomerular filter may precede the onset of low molecular weight proteinuria. In that respect, it should be noted that in diabetic patients the occurrence of a persistent microalbuminuria equivalent to that found in this study is considered as a sensitive predictor of an overt clinical nephropathy. Interestingly, the serum concentration of β_2-m also started to increase from a threshold of urinary Cd around 6 μg Cd/g creatinine, which reinforces the hypothesis of a slight reduction of the glomerular filtration rate preceding the occurrence of low molecular weight proteinuria.

The most remarkable finding in the present study is the very early increase of urinary 6-keto-PGF$_{1\alpha}$ in workers exposed to Cd. The sensitivity of this marker was such that a redefinition of the control group (subjects with Cd-U below 1 μg Cd/g creatinine) was necessary to estimate a threshold of Cd-U by logistic regression analysis. It would be premature to propose a biological threshold for exposure to Cd on the basis of this change, for which biological significance and implications in Cd nephropathy are unknown.

It can be concluded that the recent recommendation of 5 μg Cd/g creatinine in urine as the biological exposure limit for occupational exposure to Cd[5] seems justified to prevent the earliest effects of Cd on the nephron, although for most of these effects the link with the subsequent development of overt Cd nephropathy is not established. For markers that are clinically significant because they predict an overt nephropathy (low molecular weight proteinuria), the threshold of 10 μg Cd/g creatinine in urine has been confirmed.

It is not known whether the changes in urinary prostanoids result from a direct effect of Cd on their synthesis or are secondary to an effect of Cd on renal haemodynamics. Whatever the exact mechanism, an enhanced production of prostaglandins may explain the slow progression of Cd nephropathy.[25] The effects of Cd on renal prostanoids (enhanced urinary excretion of 6-keto-PGF$_{1\alpha}$ and PGE$_2$) are exactly opposite to those induced by lead. Because these prostanoids

have a vasodilative action on the renal vasculature their enhanced production might partly compensate for the reduction of glomerular filtration rate resulting from the progressive loss of renal parenchyma. This compensatory mechanism, if it adds to the adaptive changes normally occurring in remnant nephrons, can further limit the capability of measurement of glomerular filtration rate to detect the loss of functional nephrons induced by Cd.

On the other hand, prostanoids are hormones with a broad spectrum of biological activities in many organs. A recent study on cultured osteoblast like cells has suggested that Cd can stimulate bone resorption via an increased production of PGE_2.[26] If this effect occurs in humans at exposure to Cd as low as that affecting the renal synthesis of prostaglandins, this observation, in agreement with the results of the Cadmibel study,[27] suggests that environmental pollution by Cd might be a further factor contributing to bone decalcification and osteoporosis in the general population of industrialised countries.

In the present study, no reduction in RBC membrane negative charges was seen. This might be due to the degree of exposure to Cd that on average was almost 10 times lower than in workers in whom this effect was previously found.[23] Significant changes in the urinary and plasma concentrations of sialic acid were found, however, which suggest a disorder in the metabolism of sialic acid. Such an effect has been previously postulated to explain the loss of RBC surface negative charges.[23 24]

In conclusion, the application of a large battery of sensitive indicators of nephrotoxicity has allowed us to identify three main groups of thresholds of Cd-U for the development of incipient nephropathy: a threshold around 2 μg Cd/g creatinine mainly associated with biochemical alterations, a threshold around 4 μg Cd/g creatinine from which the glomerular barrier function is progressively compromised and cytotoxic effects appear in the proximal tubule, and a third threshold of 10 μg Cd/g creatinine corresponding to the onset of proximal tubular dysfunction with increased urinary excretion of low molecular weight proteins.

Appendix

CONVERSION OF UNITS

Lead 1 μg = 4·83 nmol
Cadmium 1 μg = 8·90 nmol
Mercury 1 μg = 4·99 nmol
Creatinine 1 g = 8·84 mmol

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