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Environmental epidemiological study and estimation of benchmark dose for renal dysfunction in a cadmium-polluted area in China

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Abstract

We have performed a study aimed at investigating the critical concentration of urinary cadmium (UCd) required for the development of renal dysfunction. We studied population groups (totally 790 persons) living in two cadmium exposed areas and one control area in China. UCd, was determined as an indicator of cadmium exposure and accumulation, while the concentrations of *N*-acetyl-β-D-glucosaminidase (NAG), its iso-form B (NAG-B), β2-microglobulin (B2M), retinol binding protein (RBP), and albumin (ALB) in urine were measured as indicators of the renal effects caused by cadmium. There was a significantly increased prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria and hyperALBuria with increasing levels of Cd excretion in urine. We used the benchmark dose (BMD) procedure to estimate the critical concentration of urinary cadmium in this general population. The lower confidence limit of the BMD (LBMD-05) of urinary cadmium for a 5% level of risk above the background level was estimated for each of the renal effect indicators. The BMD-05/LBMD-05 were estimated to be 4.46/3.99, 6.70/5.87, 8.36/7.31, 7.98/6.98 and 15.06/12.18 μg/g creatinine for urinary NAG-B, NAG, B2M, RBP and ALB, respectively. Our findings suggest, based on the present study, that the Lower Confidence Limit of the Population Critical Concentration of UCd (LPCCUCd-05) of tubular dysfunction for 5% excess risk level above the background may be ca. 3–4 μg/g creatinine, and that cadmium concentration in urine should be kept below this level to prevent renal tubular damage. This report is the first to use the BMD method in this field and to define the concept of critical concentration in urine.

Introduction

Renal tubular dysfunction induced by cadmium (Cd) is considered to be the critical effect of long-term human exposure to the element (WHO 1992, Nordberg 1996). Many recent studies have shown that tubular damage might develop at much lower exposure levels than previously estimated. Since cadmium is widely used, it may always pose a threat to human health. Therefore, it remains important to study the renal effects of cadmium in detail so that further risk assessment of cadmium nephrotoxicity can be undertaken. China is a developing country in which environmental pollution of cadmium from smelting and mining is dispersed widely: there are more than 30 places in China that have different sources of cadmium contamination. Thus, it was necessary to perform a detailed study of the renal effects of cadmium exposure on a general population.

In the present study, we used the benchmark dose (BMD) approach to estimate the critical concentration of urinary cadmium in a general population living in a cadmium-polluted area. For this purpose, urinary β2-microglobin (UB2M), urinary retinol binding pro-

tein (URBP), urinary *N*-acetyl-β-D-glucosaminidase (UNAG), and isoform B (UNAG-B), which are regarded as sensitive indicators of renal tubular dysfunction caused by cadmium as well as UCd were measured in the general population. Urinary albumin (UALB) was measured as an indicator of renal glomerular dysfunction caused by cadmium.

Materials and methods

Study population

The present field study, carried out in 1998, included population groups living in three areas in South East China: a highly exposed area with a cadmium content of rice (as measured 1995) of 3.70 mg/kg, a moderately exposed area with a content of 0.51 mg/kg, and a control area with a content (measured 1997) of 0.05 mg/kg. The total number of participants was 790, comprising 294 in the highly exposed area, 243 in the moderately exposed area, and 253 in the control area. It was performed according to a protocol approved by the ethics committees of Shanghai Medical University, China, and of the Medical Faculty of Umeå University, Sweden. Informed consent was obtained from both the local authority and each participant. Further background information concerning this study is available (Jin *et al.* 2002).

Collection of samples and analytical method

Urine samples, which were collected from all participants, were kept frozen at -20 °C until analysis. Urinary cadmium concentrations were measured by graphite-furnace atomic absorption spectrometry. UB2M was measured by means of RIA (radioimmunoassay) Urinary albumin was measured by ELISA. NAG isoforms were measured as described by Bernard et al. (1995). Urinary RBP was analyzed with LIA (latex immunoassay) Creatinine was measured by the Jaffe reaction method. All urinary parameters were adjusted for creatinine in urine – see the earlier report (Jin *et al.* 2002)

Benchmark dose (BMD) method

The benchmark dose (BMD) was defined by Crump (Crump 1984) as a statistical lower confidence limit to the dose that produces some predetermined increase in response rate (e.g., 1–10%). It has been suggested that the BMD could be used in risk assessment to replace the no-observed-adverse-effect level (NOAEL) or the lowest observed adverse effect level (LOAEL) in setting acceptable daily intakes (ADI) for human exposure to potentially toxic substances. More recently, Gaylor *et al.* (1998) redefined the benchmark dose as the point estimate of the dose corresponding to a specified low level of risk, and suggested that the concept of LBMD (lower confidence limit of benchmark dose) be used as a replacement for the NOAEL or LOAEL. The LBMD is identical with the original concept of benchmark dose defined by Crump. Generally, a suitable LBMD is often defined as the lower 95% confidence limit estimate of dose corresponding to a 1–10% level of risk above background (Gaylor *et al.* 1998). LBMD-05 is the lower 95% confidence limit of dose corresponding to a 5% level of risk above the background. The critical effect (the earliest adverse effect) that occurs at low-levels of urinary cadmium is close to the LOAEL. The BMD of UCd is therefore the same risk estimate as the population critical concentration of urinary cadmium (PCCUCd). In the present study, we use the LBMD procedure to estimate LPCCUCd (low confidence limit of PCCUCd). The LPCCUCd-05, which is obtained from LBMD-05, is the cadmium concentration corresponding to a 5% excess of renal dysfunction above the background. A number of statistical models have been used for fitting dose–response curves in the benchmark dose method. Quantal linear regression has been used most often to analyze quantal data, and this model has been used generally in a previous risk assessment of cadmium (Kjellström 1986). The simplest equation of this model is $\ln\{P(d)/[1-P(d)]\} = b_0 + b_1 \times d$, where P(d) is the probability of an adverse effect, d is the dose, and b_0 and b_1 are coefficients derived from the data. The LBMD can be estimated as the dose corresponding to the lower 95% confidence limit for $\ln[(P_0+\pi)/(1-P_0-\pi)]$, where P_0 is the estimate of P at dose $= 0$ and π is the excess risk specified for the BMD. In the present study, the excess risk was taken to be 0.05, so that $\pi = 0.05$.

Statistical analysis

Data were entered into a database on a microcomputer using Epi-info (version 6.04b) and analyzed by the χ^2 test and the χ^2 trend test provided in the Statcalc program of the Epi-info package. We define the cut-off points (abnormal values) for the criterion variables as the 95% upper limit values, which we collected from

the control group. For comparisons between more than two groups, we used a one-way analysis of variance (ANOVA). The analyses of regression and curve estimation were performed using the Benchmark Dose Software (version 1.3.2) designed by the US Environmental Protection Agency (EPA). Distributions of the biological measurements were normalized by logarithmic transformation. The data are expressed in terms of geometric means.

Results

Prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria, and hyperALBuria at different urinary cadmium levels

We define the normal cut-off point based on the 95% limit value in the control area. If the value found was higher than the normal cut-off point, we define the renal function as abnormal (positive). The cut-off points of UNAG, UNAG-B, UB2M, URBP, and UALB are 15.0 U/g creatinine, 4.0 U/g creatinine, 0.800 mg/g creatinine, 0.300 mg/g creatinine, and 25.0 mg/g creatinine, respectively. We calculated the prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria, and hyperALBuria in all subjects, male subjects, and female subjects at the different levels of urinary cadmium; the results are presented in Table 1 (the data for both genders are not listed). Table 1 clearly indicates that there is a significantly increased prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria, and hyperALBuria upon increasing levels of Cd excretion in urine. The increases were statistically significant in the Chi-square test for trend for each relationship between the urinary cadmium level and the prevalence of renal dysfunction. In particular, for UNAG-B, when the UCd concentration was > 2 μg/g creatinine, we found that the prevalence of hyperNAGBuria is increased significantly, relative to that for UCd < 2 μg/g creatinine. Table 1 also indicates that a significant dose–response relationship exists between cadmium exposure (UCd) and the prevalence of renal dysfunction. We estimated the dose–response curve based on the BMD procedure as presented in Figure 1.

The values of LPCCUCd-05 for different urinary indicators of renal dysfunction

The mean urinary cadmium concentrations of different UCd strata (0.52, 1.50, 3.39, 7.13, 12.24, 17.56, and 32.69 μg/g creatinine) were used in the quantal regression model to estimate the value of the LBMD-05 for different urinary indicators of renal dysfunction using the EPA program that is based on Crump's principle (Crump and Howe *et al.* 1985). The estimated parameters and the corresponding values of LBMD-05 are presented in Table 2. We observe that if urinary UNAG-B is used as an indicator of renal dysfunction, the estimated LBMD-05 of UCd is 3.7–4.0 μg/g creatinine. If UNAG is taken as an indicator of renal dysfunction, the LBMD-05 of UCd is 5.5–5.9 μg/g creatinine. The estimated values of the LBMD-05 for UB2M, URBP, and UALB lie in the ranges 4.7–8.5 μg/g creatinine, 4.9–7.6 μg/g creatinine and 11.2–12.2 μg/g creatinine, respectively. According to the P values, the outcomes of the fits of the model is better for UNAG-B, UNAG, UB2M, and URBP than it is for male UALB. The dose–response curves for each urinary indicator are displayed in Figure 1.

Discussion

Cadmium in urine is bound mainly to metallothionein and, in general, it reflects past exposure, i.e., body burden and renal accumulation. In this present study, we use the level of urinary cadmium as an estimate of the internal dose of Cd in a general population. Chronic Cd exposure gives rise to renal tubular dysfunction, the critical effect that is characterized by an increased excretion of low-molecular-weight proteins, such as B2M. UB2M has been suggested as a sensitive biomarker to test for early renal dysfunction caused by Cd (Bernard 1979). In recent years, many studies have reported that NAG activity – especially NAG isoform B activity – can be used as an even more sensitive biomarker of early renal tubular dysfunction caused by cadmium and, thus, it is often substituted for B2M (Nogawa *et al.* 1986; Kawada *et al.* 1989; Bernard *et al.* 1995). Our present study also indicates that urinary NAG and its isoform B can be more sensitive markers than B2M for renal dysfunction caused by Cd. Urinary albumin is now used widely as an index of glomerular damage. In this study, we found that ca. 38.4% renal tubular dysfunction and 10.6% glomerular damage occurs in residents of the highly polluted area; in comparison, there is a 5% prevalence of renal dysfunction in the control area. This observation indicates that long-term Cd exposure gives rise mainly to renal tubular dysfunction in the general population, which, to some extent, is accompanied by

Table 1. Prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria, and hyperALBuria at different levels of urinary cadmium in all subjects (n = 790)

Urinary cadmium	HyperNAGuria			HyperNAG-Buria			HyperB2Muria			HyperALBuria			HyperRBPuria		
(ug/g creatinine)	−	+	%	−	+	%	−	+	%	−	+	%	−	+	%
≤1.0	60	4	6.25	59	5	7.81	59	5	7.81	61	3	4.69	56	4	6.67
1.01–2.0	93	9	8.82	96	6	5.88	95	7	6.68	96	6	5.88	88	9	9.28
2.01–5.0	246	25	9.23	237	34	12.55	255	16	5.90	259	12	4.43	243	14	5.45
5.01–10.0	140	23	14.11	130	33	20.25	150	13	7.98	154	9	5.52	141	19	11.88
10.01–15.0	69	18	20.69	60	27	31.03	70	17	19.54	79	8	9.20	72	14	16.28
15.01–20.0	27	15	35.71	23	19	45.24	35	7	16.67	40	2	4.76	34	7	17.07
>20	30	31	50.82	15	46	75.41	32	29	47.54	47	14	22.95	30	30	50.00
Linear trend test	$\chi^2 = 87.49$ p= 0.000			$\chi^2 = 158.52$ p= 0.000			$\chi^2 = 94.85$ p= 0.000			$\chi^2 = 29.44$ p= 0.000			$\chi^2 = 91.99$ p= 0.000		

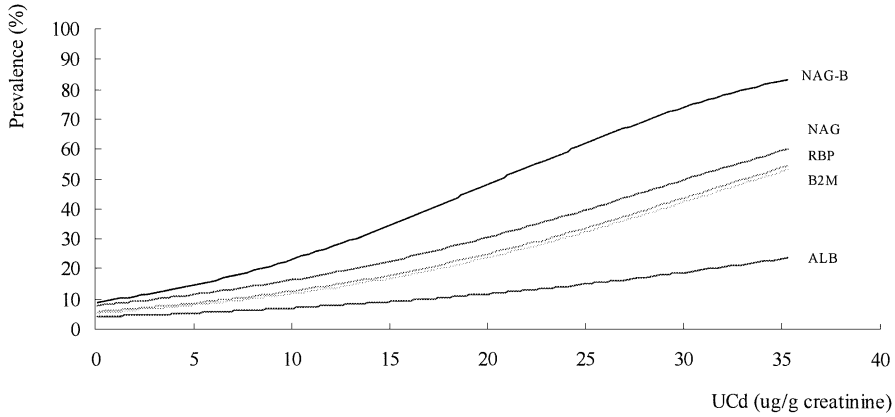

Fig. 1. Dose-response curves of UCd and urinary NAG-B, B2M, RBP and ALB.

glomerular damage. In our study, we used the critical concentration of cadmium in urine to be an indicator of the critical concentration of cadmium in the kidney cortex.

A number of studies of the dose–response relationship between cadmium exposure and renal effects have been carried out for both occupational and environmental exposure to cadmium (Nordberg 1992). In the 1970s, it was suggested that a urinary cadmium concentration of 10 μg/g creatinine is a safe level; this value corresponds to a kidney cortex concentration of 200 mg/kg (Bernard *et al.* 1979; Friberg *et al.* 1986). While more sensitive methods and other early indicators of renal dysfunction have now become available, renal dysfunction has also been detected at relatively low levels of cadmium during the past 12 years. The Cadmibel study, an important and frequently cited study of the effects of cadmium on the general population, indicated that a significant proportion (10%) of a population showed evidence of renal damage at UCd concentrations exceeding 2–4 μg/g creatinine (Buchet *et al.* 1990; Bernard *et al.* 1992). In an analysis of several studies from Japan, Nogawa demonstrated that a lifelong cumulative oral intake of ca. 2 g of cadmium would cause B2Muria; the corresponding UCd values were 3.8 μg/g creatinine for men and 4.1 μg/g creatinine for women (Nogawa *et al.* 1992). A recent study from China (Jin *et al.* 1999) also demonstrated a significantly increased excretion of urinary NAG isoform B in subjects exposed environmentally to cadmium at UCd concentrations of 2–5 μg/g creatinine. As pointed out by Järup *et al.* 1998 in a review of the health effects of cadmium, all recent findings suggest that the critical concentrations of cadmium in urine and in the kidney have previously been overestimated.

Table 2. LBMD Estimates of UCd (μg/g creatinine) for urinary indicators of renal dysfunction by 95% of cut off point

Indicators		b_0	b_1	BMD-05	LBMD-05 (LPCCUCd-05	P values*
	Female	−2.416	0.084	6.36	5.46	0.83
NAG	Male	−2.483	0.072	7.74	5.83	0.78
	Total	−2.449	0.081	6.70	5.87	0.67
	Female	−2.322	0.118	4.24	3.70	0.55
NAG-B	Male	−2.339	0.104	4.88	3.98	0.87
	Total	−2.323	0.112	4.46	3.99	0.51
	Female	−3.192	0.087	9.98	8.47	0.49
B2M	Male	−2.610	0.103	5.86	4.74	0.79
	Total	−2.871	0.085	8.36	7.31	0.50
	Female	−2.811	0.076	9.03	7.63	0.51
RBP	Male	−2.859	0.118	5.99	4.87	0.48
	Total	−2.783	0.084	7.98	6.98	0.54
	Female	−2.993	0.053	14.42	11.31	0.70
ALB	Male	−3.525	0.064	16.72	11.18	0.10
	Total	−3.172	0.057	15.06	12.18	0.78

Model: $\ln\{(P(d)/[1-P(d)]\} = b_0 + b_1 \times d$
Excess risk at BMD is 0.05.
*P values were obtained from the chi-square test, with the Pearson goodness of fit test, if P> 0.05 then the equation is a good fit.

In the present study, we used the BMD procedure to calculate the PCCUCd based on population data of UNAG, UNAG-B, UB2M, URBP, and UALB as indicators of renal dysfunction. The LPCCUCd-05 is the lower confidence level of the estimated 5% level of renal dysfunction above the background, as estimated based on the dose–response model, and is identical to the LBMD-05 according to the 'Benchmark dose' terminology. Our results suggest that there are different values of LPCCUCd-05 depending on which urinary indicator of renal tubular dysfunction is used. The LPCCUCd-05 for urinary NAG-B gave the lowest value (ca. 3.7 μg/g creatinine), which supports the view that NAG-B is a very sensitive and early biomarker of renal tubular dysfunction induced by cadmium. In brief, the value of LPCCUCd-05 of renal tubular dysfunction lies in the range 4–8 μg/g creatinine based on a comprehensive analysis of the four urinary indicators (UNAG, UNAG-B, UB2M, and URBP). This range implies that there is an increased 5% prevalence of renal tubular dysfunction in a general population having a level of UCd of 4–8 μg/g creatinine. A 'best guess' of the prevalence of tubular effects in the general population (Järup *et al.* 1998), which was based on all the available data published prior to mid-1997, suggested that the PCC-05 of cadmium in the renal cortex, at which the 5% effect can be detected, is ca. 95 mg/kg; the corresponding UCd is ca. 4.75 μg/g creatinine. Also, it was suggested that cadmium levels in the kidneys and in urine be kept below 50 mg/kg and 2.5 μg/g creatinine, respectively, to prevent renal tubular damage that can proceed to clinical disease and, perhaps, contribute to early death; these limits imply that the previous estimated value of $PCC\text{-}_{10}$ (180–200 mg Cd/kg) was significantly overestimated. Further improvements in analytical methods and the application of sensitive indicators of early renal dysfunction, when combined with our present findings, suggest, however, that the UCd level at which the lowest detectable renal effect should be observed occur at much lower concentrations than previously evaluated.

Finally, a different model may have to be used with regard to the different data or purposes. Generally, the quantal linear logistic regression model is used in the BMD procedure and also has been used often in previous risk assessments of cadmium. Furthermore, the present results indicate that this model provides a good fit to the present data. Therefore, in this study we selected the quantal linear logistic regression model – not the general linear model – to estimate the critical concentration of UCd. This report

is the first in which the BMD procedure has been used to estimate the UCd critical concentration of renal dysfunction in a general population exposed to cadmium. The present estimated values of LPCCUCd-05 of renal tubular dysfunction in a general population exposed to cadmium is in accordance with the lowest limit value of UCd concentration evaluated in other recent studies (Järup *et al.* 1998).

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