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Lack of reversal effect of EDTA treatment on cadmium induced renal dysfunction: a fourteen-year follow-up

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Abstract

The aim of this study was to evaluate the effect of ethylenediaminetetraacetic acid (EDTA) on cadmium (Cd) induced renal dysfunction. Seventeen workers (14 males, 3 females) were diagnosed with occupational Cd poisoning in 1986. These individuals had between 7 to 39 years of Cd exposure. From 1986 to 1999, patients received periodic EDTA therapy as part of their follow-up, all at the same hospital. Levels of urinary cadmium (UCd) and urinary beta2-microglobulin (B2M) were measured before and after each annual EDTA treatment period. Renal dysfunction was defined as urinary B2M $\geq$ 0.8 mg/g Cr (creatinine). In these workers, patients with UCd level higher than 10 μg/g Cr in 1986 had abnormal B2M excretions ($\geq$0.8 mg/g Cr) or trended to have abnormal B2M levels during the treatment period. However, in subjects with UCd concentration lower than 10 μg/g Cr in 1986, their urinary B2M excretions either remained normal (<0.8 mg/g Cr) or returned to normal during the treatment period. The prevalence of renal dysfunction increased during the follow up period regardless of whether UCd levels increased or not, indicating a progressive renal dysfunction despite removal from Cd exposure. Our results suggest that reversibility of renal dysfunction caused by Cd related to the level of Cd exposure at the time of removal from exposure: renal dysfunction could be reversed if initial UCd < 10 μg/g Cr, but was irreversible when UCd > 10 μg/g Cr. Repeated examinations on these 17 Cd exposed workers from 1986 to 1999 also revealed that periodic administration of EDTA had no beneficial effects on chronic Cd-induced renal dysfunction.

Introduction

Cadmium (Cd) is widely used in industry and is well known nephrotoxic metal. It can enter the human body orally or via inhalation into the respiratory tract, and accumulates in many tissues, with the highest concentration found in the kidney. The half-life of Cd in the human body is long (10–30 years), so that its accumulation effectively can be lifelong. Cd is excreted in the urine. Thus, the level of urinary cadmium (UCd) can be used as a marker of Cd exposure level and total body burden (WHO/IPCS, 1992). Long term and chronic exposure to Cd mainly gives rise to renal tubular dysfunction with increasing excretion of low molecular weight (LMW) protein (such as β2-microgolbulin, B2M) in the urine, a well known early sign of renal tubular dysfunction caused by Cd exposure (Friberg *et al.*, 1985).

Ethylenediaminetetraacetic acid (EDTA) has been widely used for the past 4 decades as an antidote for the treatment of acute and chronic metal poisoning because it can chelate metals in the human body to form a complex, promoting the excretion of metals in urine (Andersen, 1984). Administration of EDTA significantly increased the urinary elimination of Cd (Klaassen *et al.*, 1984); but the mobilization of Cd from other organs to the kidneys by EDTA may increase Cd content in the kidneys, resulting in a potential risk of nephrotoxicity. To examine the effect

of EDTA therapy on the progression of renal insufficiency in patients with Cd poisoning, we followed prospectively the changes of UCd and urinary B2M levels among a group of Cd exposed workers, who had been periodically treated with EDTA during a 14 year period in Nanning, China.

Subjects and methods

Subjects

This study was approved by the Human Institutional Review Board at Guangxi Worker's Hospital. Seventeen workers (Male: 14, female: 3), all worked in a Cd smelter in which adverse health effects of Cd exposure on workers have been found previously by us and others (Zhuo *et al.*, 1989; Wu *et al.*, 2000; Bo *et al.*, 2000), were diagnosed with occupational Cd poisoning in 1986. All subjects consented to be treated and followed in this study from 1986 to 1999. The original diagnoses of Cd poisoning were established on the basis of occupational exposure history, increased UCd, enhanced urinary B2M, according to the China Diagnostic Criteria of Occupational Cd Poisoning. These workers received an annual period of intravenous EDTA treatment (0.5 g EDTA/day for three days) at Guangxi Worker's Hospital for 11 times from 1986 to 1999 (treatment did not occur between the years, 1992–1994). UCd and urinary B2M were measured before and after each EDTA treatment period for every patient. In 1986, the average age of subjects was 60.25 (37–75) years, and the mean exposure time to cadmium was 17.7 (7–39) years. Some, but not all, individuals were removed from their Cd exposure environment during the course of their treatment and follow-up period. To determine the cut-off point for renal dysfunction (hyperB2Muria), 92 workers (70 males, 22 females) without a cadmium exposure history were recruited as controls. No significant differences in age, gender, and living conditions were found between the exposed group and the control group.

Sampling and analysis

Twenty four-hour urine samples were collected from all workers before and after each EDTA treatment period. All urine sample containers were immersed in nitric acid fluid for 48 h to eliminate contaminated metals before they were used for collecting urine samples. The urine samples were stored at $-20\,^{\circ}C$ until analysis. Each sample was divided into two parts immediately after collection. One part was acidified with concentrated nitric acid and was used for assay of Cd; the other part was used for the measurement of B2M. UCd was determined by atomic absorption spectrometry (Perkin-Elmer, USA). Urinary B2M was measured using the RIA method (Evrin *et al.*, 1971). Kits were purchased from the China Institute of Atomic Energy, Beijing. Urinary creatinine was assayed using commercial kit (Jaffe reaction method). All values of UCd and urinary B2M were adjusted for urinary creatinine. To ensure the accuracy of analysis, quality control procedures were applied to all measurements. Briefly, a standard curve was generated using known concentrations of the metal, protein and creatinine of interest. The standard materials of B2M and creatinine were provided by the kit manufacturers, and standard Cd was purchased from the Standard Material Center of China. Values for samples were determined using the linear portion of the curves for standards run in parallel with each batch of assays. In addition, a sample of blind standard material was included in each batch of analyses. When the value of the standard material was within the expected range, the result of the samples were considered acceptable.

Statistical analysis

Urinary B2M and Cd concentrations were expressed as mg/g creatinine and μg/g creatinine respectively. Data were entered in a database on a microcomputer using Epi-info (version 6.04b). The cut-off point for renal dysfunction (hyperB2Muria) was found to be 0.8 mg/g Cr, defined as the 95% upper limit values from the control group (Wu *et al.*, 2000). Statistical analysis was performed using SPSS for Windows 8.0 software (Chicago, Illinois). No statistical analysis was carried out among various subgroups because the number of subjects in each subgroup was small.

Results

Demographic information

In this group of 17 workers, exposure was between 7 and 39 years. Environmental monitoring data showed that mean air Cd concentrations in the workplaces at this Cd smelter in 1960, 1970, 1982, and 1987 were 1.211, 0.190, 0.059, 0.286 mg/m^3 respectively (Zhuo *et al.*, 1989), which were higher than current China permissible concentration-time weighted average (PC-TWA, 0.01 mg/m^3). Among 17 workers, 7 workers

Table 1. Mean pre-treatment and post-treatment UCd levels.

Group	n	Age (year) and range	Total Cd exposure duration (year)	Years since cessation of Cd exposure	Mean UCd (μg/g Cr)[a] of Pre EDTA-treatments	Mean UCd (μg/g Cr) of Post EDTA-treatments
A	3	51[a] (44–60)[b]	9.3 (7–12)	5.7 (0-12)	6.7 (2–9.8)	8.8 (2–23.2)
B	5	61 (48–75)	15.0 (10–18)	10.4 (0–27)	5.8 (2.2–9.6)	6.8 (2.6–16)
C	7	58 (37–71)	18.4 (12–26)	5.7 (0–14)	9.9 (1.8–32)	11.9 (3.4–35.0)
D	2	71 (70–72)	28 (17–39)	16 (15–17)	15.3 (4.8–29.0)	19.9 (4.9–39.5)

[a]The average level of UCd of pre and post EDTA treatment in 14 year follow-up.

Table 2. Mean UCd and beta2-microglobulin levels, and prevalence of abnormal levels in 1986 (start of study) and in 1999 (end of study).

Group	Year	UCd(ug/g Cr)		Urine B2M(mg/g Cr)	
		Mean (range)	Prevalence (%) of abnormality	Mean (range)	Prevalence (%) of abnormality
decreased-UCd group	1986	7.2 (2.9–29.0)	25.0	0.26 (0.18–2.50)	33.3
($n = 12$)	1999	4.7 (2.2–15.5)	0.8	1.21 (0.13–2.56)	58.3
increased-UCd group	1986	6.0 (4.0–10.0)	20.0	0.13 (0.12–0.38)	0.0
($n = 5$)	1999	9.0 (5.4–34.6)	40.0	0.42 (0.19–2.05)	40.0

UCd > 10 μg/g Cr was considered as abnormal;
B2M ≥ 0.8 mg/g Cr was considered as abnormal.

have had 10–29 years of smoking histories. Although smoking is considered an important source of cadmium exposure, no tobacco is produced in this area and only commercial cigarettes are smoked. The average cadmium concentration of eight different brands of commercial cigarettes, which are generally used by local residents, was determined and found to be 1.50 mg/kg in 1995 (Jin *et al.*, 1999). For a person who smoked 10 g of tobacco per day, i.e., 20 cigarettes per day, for 25 years, the uptake of Cd from smoking was calculated to be about 1.05 mg (estimated as described by Cai *et al.*, 1998), which is much lower than the cadmium uptake from the work environment. Because of this reason, smoking was not considered to have a significant influence on the value of total cadmium uptake in the present study.

The mean pre-treatment UCd was 7.0 (2.9–29.0) at the time of diagnosis in 1986. Mean pre-treatment urinary levels of B2M in 1986 were 0.47 mg/g Cr (0.12–2.50). The proportion of workers with urinary B2M greater than 0.8 mg/g Cr was 23.5% in 1986. At the time of diagnosis in 1986, 12 workers had already been removed from Cd exposure. During the course of treatment and follow-up an additional 5 workers were removed from Cd exposure.

Changes in UCd levels before and after EDTA treatment in Cd exposed workers

The 17 workers with occupational Cd poisoning were divided into four subgroups according to the change in their renal function during the treatment period: group A (renal dysfunction recovered); group B (renal function remained normal); group C (renal dysfunction developed during follow-up period); group D (renal function was always abnormal). The demographics of Cd exposed workers and the changes of UCd before and after EDTA treatment, by subgroup, are shown in Table 1. The average age of workers in group A was the youngest and exposure time was the shortest. The average pre-treatment UCd levels in group B was the lowest. Group B had no annual mean UCd levels exceeding 10 μg/g Cr during the entire treatment period. The highest UCd levels before and after EDTA treatment were observed in group D, and the renal function of two workers in this group was always abnormal from the beginning to the end of EDTA treatment.

These two patients had the longest mean Cd exposure time, but also the longest time of removal from Cd exposure. In all four subgroups, mean UCd level after EDTA treatments during 14 year follow-up were higher than the pretreatment mean value.

Comparison of the levels of mean UCd and urinary B2M in Cd exposed workers between first (1986) and last EDTA treatments (1999)

The mean value of pre- and post-EDTA treatment levels of UCd and urinary B2M in 1986 (beginning of study treatment period) and 1999 (end of study treatment period) are shown in Table 2. Cd exposed workers were divided into two groups: (A) patients with an increase in UCd over the treatment period (UCd-increased group), where the mean UCd levels at the end of the treatment period (1999) were higher than initially (1986); and (B) patients with decreased UCd over the treatment period (UCd-decreased group), where UCd levels in 1999 were lower than that in 1986. Both the mean level and prevalence of hyperB2M in 1999 was higher than that in 1986 in both UCd-increased and UCd-decreased groups. In the UCd-increased group, all 5 workers continued to be exposed to Cd throughout the treatment and follow-up period until 1991, reflecting increasing total body burden of Cd.

The prevalence of hyperB2Muria in subjects with different exposure removal times

Table 3 shows that the prevalence of hyperB2Muria in 1999 in Cd exposed workers was always higher than in 1986, regardless of how long workers were removed from Cd exposure, suggesting that the prevalence of renal dysfunction increased during the entire EDTA treatment period.

The changes in the prevalence of hyperB2Muira between 1986 and 1999 in workers with different UCd levels

In order to analyze the relationship between the prevalence of hyperB2Muria and UCd level, Cd exposed workers were divided into two groups (UCd $\geq$ 10 μg/g Cr and UCd $<$ 10 μg/g Cr) according to the initial UCd level examined in 1986. Mean changes in UCd levels and prevalence of hyperB2Muria between 1986 and in 1999 are shown in Figures 1 and 2, respectively. The prevalence of hyperB2Muira decreased during the follow-up period in the group with

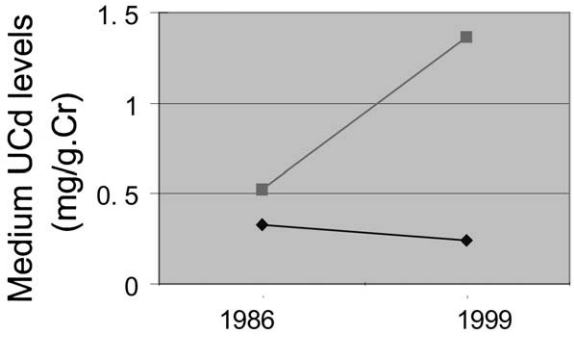

Fig 1. Changes in mean urinary B2M between 1986 and 1999 in subjects with UCd $<$ 10 μg/g Cr in 1986 (♦, $n = 9$), and in subjects with UCd $>$ 10 μg/g Cr in 1986 (■, $n = 8$).

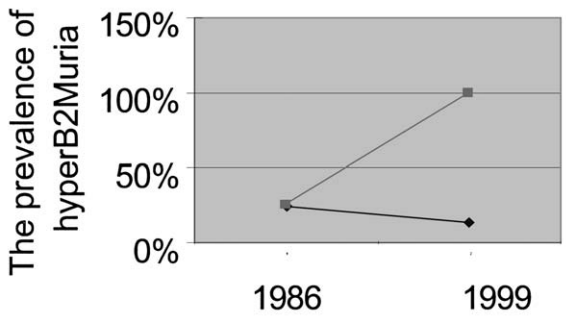

Fig 2. Changes in prevalence of hyper B2Muria between 1986 and 1999 in group1 (♦, $n = 9$, mean UCd $<$ 10 μg/g Cr in 1986), and in group 2 (■, $n = 8$, mean UCd $>$ 10 μg/g Cr in 1986).

UCd $<$ 10 μg/g Cr, but increased in workers with UCd $\geq$ 10 μg/g Cr, indicating that Cd-induced renal dysfunction in workers with UCd less than 10 μg/g Cr could be reversed.

Discussion

The reversibility of renal dysfunction in Cd-exposed workers was first investigated by Friberg and his coworkers in 1952 (Friberg *et al.*, 1952), who found that proteinuria in Cd-exposed workers lasted for many years after the cessation of exposure. In practical terms, Cd-induced renal dysfunction was irreversible. Afterwards, a number of studies on the prognosis of Cd-induced renal dysfunction have been carried out, but the conclusions have been controversial (Tsuchiya 1976; Itawa *et al.*, 1993). Our results showed that the reversibility of Cd induced renal dysfunction was related to the total body burden of Cd exposure as measured by UCd. In subjects with an initial UCd level $<$10 μg/g Cr, renal dysfunction was potentially reversible, while in patients with UCd $>$ 10 μg/g Cr

Table 3. The prevalence of hyperB2Muria in workers after various durations since cessation of Cd exposure.

Years since cessation of Cd exposure	N	HyperB2Muria in 1986		HyperB2Muria in 1999	
		N	Prevalence (%)	N	Prevalence (%)
0–<5	6	1	15.6	2	33.3
5–<10	5	1	20.0	3	60.0
10–<15	2	0	0.0	1	50.0
>15	4	2	50.0	3	75.0

renal dysfunction was generally irreversible. Our results also indicated that EDTA has no beneficial effect on Cd induced renal tubular dysfunction, as measured by B2M in the urine. On the contrary an effect as increased excretion of B2M was seen indicating an increased tubular dysfunction.

Since previous studies confirmed that UCd reflects the body burden of internal Cd (Lauwerys *et al.*, 1983), UCd was measured as a biomarker of Cd exposure in the present study. Dose-response relationships between UCd and urine B2M has been well documented (Kido *et al.*, 1993; Elinder *et al.*, 1985). Low molecular weight proteinuria, such as B2Muria, is a main characteristic of renal dysfunction caused by Cd exposure (Bernard *et al.*, 1992). In addition, urinary B2M concentration correlates closely with the results of four other renal tubular function tests: renal glucosuria, urinary total amino nitrogen excretion, uric acid clearance, and the percentage tubular reabsorption of phosphate (%TRP) (Shitomi *et al.*, 1981). Therefore, we utilized urinary B2M concentration as a surrogate marker of overall tubular renal function in the present study. The average pre-treatment level of UCd in workers whose renal function was either normal or had returned to normal during EDTA therapy, was lower than 10 μg/g Cr. Conversely, UCd was higher than 10 μg/g Cr in workers whose renal function were abnormal or became abnormal during EDTA treatment (Table 1). Our observation suggested that UCd levels (i.e. total body burden of Cd) might play a crucial role in the reversibility of renal dysfunction. The prevalence of hyperB2Murina at the end of the treatment and follow-up period (1999) was higher than the initial measurements in 1986 in both UCd-increased and UCd-decreased groups (Table 2), suggesting that despite many years of EDTA treatment, renal dysfunction persisted after the cessation of Cd exposure.

In this study, mean Ucd levels after EDTA treatment during 14 year follow-up were higher than the pretreatment mean value, indicating an increased elimination of Cd from the body by EDTA therapy. However, urinary B2M concentrations generally increased in our study subjects, suggesting a persistent renal dysfunction. These conflicting results might be associated with the EDTA treatment. It has been reported that calcium EDTA has the potential for nephrotoxicity despite its chelating ability to cadmium (Klaassen *et al.*, 2001). Moreover, there is evidence that use of EDTA as chelator in Cd exposure may cause a redistribution of cadmium in the body (Friberg *et al.*, 1979). For example, in toads administered Cd, EDTA therapy caused striking reduction in the cadmium content of all organs and tissues except the kidneys (Hilmy *et al.*, 1986). Because no control group was selected in the follow-up study, it was impossible to examine whether the increased prevalence of hyperB2Muria during the observation period was larger than that expected from aging alone. It should be pointed out, however, that age-related increase in urinary B2M excretion usually occurs only in individuals who are over 70 year of age, and the upper limit for urinary B2M concentration does not exceed 1500 μg/g Cr in non-Cd exposed populations (Kowal *et al.*, 1983). Thus the increase in hyperB2Muria in the present study cannot be explained by the aging process alone.

Does the duration of stopping Cd exposure promote the recovery of renal dysfunction in Cd exposed workers? The present study showed that renal dysfunction did not recover, but instead persisted and worsened even at 10 years (or greater) after cessation of exposure. Our results are in agreement with the studies of Roels (Roels *et al.*, 1982) and Stewart (Stewart *et al.*, 1981) who found that Cd-induced renal lesions were irreversible, albeit with slower renal function deterioration in workers removed from ex-

posure. Two possible explanations of irreversibility of renal dysfunction might be: (A) The half-life of Cd in the human body is between 10 and 30 years, whereby accumulated Cd would produce continuously toxic effects on renal tubular function after stopping exposure; (B) Renal dysfunction will become more difficult to reverse as the subjects aged. Nevertheless, 3 of 17 Cd exposed workers who were detected with renal dysfunction in 1986 reverted to normal tubular renal function in 1999. Thus Cd-induced renal dysfunction is not irreversible in all circumstances. The fact that the recovery of renal dysfunction was related to UCd levels in 1986 indicated that total Cd exposure level was a crucial factor in determining the reversibility of renal dysfunction in Cd-exposed workers. Of note, UCd can reflect not only the level of Cd exposure and body burden, but also renal tubular function to some extent. In 1979, it was suggested that a UCd concentration of 10 μg/g Cr would be a safe level; this corresponded to a renal cortex concentration of 200 mg/kg (Lauwerys *et al.*, 1979). But more recent studies showed that renal dysfunction may happen at relatively low levels of Cd. Nogawa *et al.* showed that a lifelong intake of about 2 g of Cd was associated with hyperB2Muria, while the corresponding UCd levels were 3.8 μg/g Cr for men and 4.1 μg/g Cr for women (Nogawa *et al.*, 1992). Our previous study also demonstrated that a significantly increased excretion of urinary NAG isoform B in subjects environmentally exposed to Cd at UCd concentrations of 2–5 μg/g Cr (Jin *et al.*, 1999). It suggested that the damage of renal tubular in workers with more than 10 μg/g Cr of UCd might be too serious to reverse.

Although EDTA enhanced the excretion of cadmium, repeated periodic administration of EDTA did not ameliorate Cd-induced renal dysfunction in the present study. The mechanisms by which Cd is toxic are currently incompletely understood, and lipid peroxidation, proteinuria, glucosuria, enzymatic cessation, and catalytic cycle disruption may all be involved in Cd toxicity (Kelley *et al.*, 1999). Successful therapies may be based on combinations of multiple methods, such as chelating agents, metallothionein inducers, vitamin and hormone treatment, Cd antagonism by other metals, and anti-oxidants. The duration of treatment course, dose, frequency, and the extent and severity of cadmium exposure levels may also influence the curative effect of EDTA therapy on cadmium induced renal dysfunction.

In conclusion, we found no curative effects of periodic administration of EDTA on Cd induced renal dysfunction. Our results also confirmed previous findings that the reversibility of renal dysfunction caused by Cd exposure is related to UCd levels, an indicator of total Cd exposure. Since the reversibility of renal dysfunction caused by Cd may be affected by many factors, and this study was only done on 17 Cd-exposed workers, further study of the mechanisms involved in the renal dysfunction resulting from Cd exposure should assist in developing new strategies for preventing and treating Cd induced renal dysfunction.

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