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Toxicology

Chronic exposure to low-level cadmium induced zinc-copper dysregulation

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ABSTRACT

Background and objectives: Exposure to cadmium (Cd) has been associated with aberrant zinc and copper homeostasis. This study investigated if Cd exposure impairs renal reabsorption of metals.
Methods: Renal tubular reabsorption of metals were calculated from urine to serum metal ratios and analyzed for an independent association with Cd exposure levels, using data from 100 men and 100 women, aged 16–60 years.
Results: The smoking prevalence was 30% in men and 0% in women. The male and female means (SD) for urine Cd were 0.54 (0.43) and 0.62 (0.43) μg/g creatinine. The mean (SD) for fractional zinc reabsorption was 77.2 (23) % in men and 87.7 (13.3) % in women, while the copper reabsorption was 100% in both men and women. Lower zinc reabsorption levels were associated with higher Cd exposure ($P < 0.001$), higher serum copper to zinc ratios ($P = 0.007$) and higher tubular impairment levels ($P = 0.024$). Reduced zinc reabsorption was particularly severe in smokers as those with high Cd exposure had 44.9% and 37.2% ($P < 0.001$) lower zinc reabsorption than those with low and moderate exposures. The mean zinc reabsorption in male non-smokers with high Cd exposure was 25.8% ($P < 0.001$) and 18.2% ($P = 0.003$) lower than those with low and moderate exposures, while the corresponding figure for female non-smokers was 17% ($P < 0.001$), and 12.8% ($P = 0.013$), respectively.
Conclusions: This is the first report demonstrating Cd-dose dependent reduction in renal zinc reabsorption and high serum copper to zinc ratios.

1. Introduction

Cadmium is an environmental toxicant of continuing public health concern worldwide [1–4]. For the general population, diet is the main Cd exposure source, while cigarette smoke is an additional Cd source for smokers [1,4]. By the total diet studies, estimated levels of dietary Cd intake in the average consumer are 8–25 μg/day, 40–60% of which coming from staple foods such as rice, potatoes and wheat, while shellfish, crustaceans, molluscs, offal and spinach form the remaining dietary sources of Cd [1]. Currently, Cd exposure has been associated with a wide range of ill health including chronic kidney disease (CKD), hypertension, diabetes, macular degeneration, and cancer of various sites [1–3]. However, the most frequently reported Cd toxicity in non-occupational exposure situations is related to kidneys, especially the injury to and impairment of the proximal tubular epithelial cells that actively reabsorb and concentrate Cd from the glomerular filtrate [2,5]. Cd-linked tubular impairment have been evaluated by urinary levels of small solutes (glucose, amino acids, calcium, phosphorus) and the low-molecular weight proteins, notably α1-microglobubin (α1-MG) and β2-microglobulin (β2-MG) [5–9], while urinary Cd excretion is an indicator of long-term exposure [10,11].

Increased urinary excretion of calcium, bone demineralization and an increased risk of osteoporosis and fracture are considered to indicate renal tubular toxicity of Cd exposure [8,12–14], while an increased urinary excretion of glucose and calcium together with reduced renal reabsorption of phosphorus indicate adverse renal outcomes in chronic high-dose exposure situations [15–17]. Of interest, reduced serum zinc levels and altered homeostasis of copper and zinc in human livers and kidneys have also been reported in low-dose exposure conditions [18–20], but the molecular basis for these exposure outcomes is not

Abbreviations: β2-MG, β2-microglobulin; Cd, cadmium; Cu, copper; FAO/WHO, Food and Agriculture Organization/World Health Organization; Zn, Zinc

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well understood. Presently, there is increasing knowledge on the transporter systems that are expressed in renal tubular cells to allow reuptake of the nutrients and essential metals that would otherwise be lost through urinary excretion [21–24]. Of relevance, experimental animal studies have implicated zinc transporters, namely ZIP8, ZIP10, and ZIP14 in renal Cd uptake [23,24]. In addition, interaction of Cd ions and zinc transporters has been suggested in the studies that used nephron microinjection and proximal tubule perfusion techniques [25,26]. In light of these knowledges and a well-established role of kidneys in the homeostasis of metals and nutrients, notably zinc and glucose [27,28], we sought evidence for adverse effects of Cd exposure on renal reuptake of zinc and copper. Accordingly, we evaluated an association between Cd exposure and fractional reabsorption of zinc and copper in relationship to age, gender, body mass index (BMI), smoking status and levels of tubular impairment, assessed by urinary β2-MG levels.

2. Materials and methods

2.1. Study subjects

The Institutional Ethical Committee, Chulalongkorn University Hospital, Chulalongkorn University, Bangkok, Thailand approved the study protocol. A total of 200 persons, 100 women and 100 men, 16–60 years of age, formed our study subjects. They were participants in a population-based study, conducted in Bangkok, Thailand as detailed previously [29]. In brief, all participants were Thai nationals who lived in residential areas surrounding Bangkok, when the study was undertaken. They took part in the study after giving informed consent. All were apparently healthy and had no history of exposure to metals in the workplace.

2.2. Collection of blood and urine samples and metal reabsorption assessment

One 15-mL blood sample was collected from each subject 1 h after drinking of 300 mL water following an overnight fasting. Urine samples were collected over 3 h after the blood sample was drawn. These procedures of collection of blood and urine samples 3 h apart enabled us to determine the renal zinc and copper reabsorption together with creatinine secretion based on the principle that the kidney has the capacity secrete waste products that require elimination from the body. Under normal physiologic conditions, the concentrations of waste products such as creatinine in urine are greater than in serum so that the urine to serum concentration ratio of a given waste product is greater than 1. In distinction from waste products, concentrations of essential metals such as zinc and copper in urine are fractional to those in serum, suggesting active reuptake of these metals. Thus, the renal secretion of creatinine is based on the equation; [(1 − serum to urine creatinine concentration ratio) × 100%], while the fractional reabsorption of a given metal can be calculated with the equation; [(1 − urine to serum metal concentration ratio)/(1 − serum to urine creatinine concentration ratio) × 100%] [15–17,30].

2.3. Assessment of exposure and outcomes

Kidney effects were evaluated by urinary excretion levels of β2-MG, metals and creatinine, while Cd exposure was evaluated by urinary and serum Cd levels. The copper and zinc status was based on serum concentrations. The urinary β2-MG assay was based on the latex immunoagglutination method (LX test, Eiken 2MGII; Eiken and Shionogi Co., Tokyo, Japan) [31,32]. The urine creatinine assay was based on the Jaffe's reaction, using the automated system available in the Chulalongkorn University Hospital Laboratory. The levels of metals (Cd, Cu, Zn) in urine and serum samples from study subjects were determined with the inductively-coupled plasma/mass spectrometry (ICP/MS, Agilent 7500, Agilent Technologies), calibrated with multi-element standards (EM Science, EM Industries, Inc., NJ, U.S.). The accuracy and precision of the metal analysis were evaluated with the reference whole blood (Seronorm™ Trace Elements, Norway), and the reference urine (Lyphochek®, Bio-Rad, Australia). The recommended Cd, Cu and Zn concentrations were 0.67–0.76, 620–638, and 5051–5336 μg/L in the low-range whole blood control, while the corresponding metal concentrations in the high-range whole blood control were 5.4–7.2, 675–697, and 5351–5642 μg/L, respectively. The recommended concentrations of Cd, Cu and Zn in the low-range urine control were 4.9–7.4, 7.7–12.8, and 571–856 μg/L, while the corresponding metal concentrations in the high-range urine control were 8.9–13.4, 28–46, and 940–1411 μg/L, respectively. Aliquots of whole blood and urine controls, serum and urine samples from the subjects were digested in distilled HNO3 equilibrated on a boiling water bath for 2 h [10,20]. All containers used in specimen collection and preparations were metal-free, while the HNO3 (69%, w/v) was distilled to achieve the highest purity possible. Our analysis of Cd, Cu and Zn concentrations in the low-range whole blood control gave the mean (SD) of 0.80 (0.08), 630 (7.5) and 5215 (122) μg/L, while the mean (SD) of Cd, Cu and Zn concentrations in the high-range whole blood control were 7.25 (0.06), 686 (9) and 5534 (127) μg/L, respectively. For the low-range urine control, our analysis gave the mean (SD) of 6.20 (0.47), 7.38 (1.38), 748 (47) μg/L for Cd, Cu and Zn, while the mean (SD) of Cd, Cu and Zn concentrations in the high- range urine control were 11.30 (0.68), 34.79 (2.70), and 1203 (100) μg/L, respectively. The coefficients of variation of repeated analysis of Cd, Zn and Cu in the whole blood and urine controls were within an acceptable range (< 5%). The urine and serum samples containing Cd levels below the limit of detection (LOD) of 0.05 μg/L were assigned as the LOD divided by the square root of 2.

2.4. Statistical analysis

The SPSS statistical package 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyze data. The Mann-Whitney *U* test was used to compare mean differences between women vs. men, and smokers vs. non-smokers, while the Chi-square test was used to compare differences in the prevalence data. The Kolmogorov-Smirnov goodness of fit to test for conformity to normal distribution of measured variables. Distribution of the variables was examined for skewness and those showing right skewing were subjected to logarithmic transformation before analysis, where required. A linear regression model analysis was used to identify the determinants of zinc reabsorption, and to evaluate the strength of associations between zinc reabsorption and its determinants. The generalized linear model (GLM) was used to estimate effect size of Cd exposure after adjustment for covariates and interaction. P values ≤ 0.05 for a two-tailed test was considered to indicate statistical significance.

3. Results

3.1. Descriptive characteristics of study subjects

The mean age for participants was 39.28 years. On average, women were 6 years older than men ($P < 0.001$). Thirty percent of the men were current or former smokers, while all the women were non-smokers ($P < 0.001$) (Table 1). The mean body mass index (BMI) in men of 23.24 kg/m^2 was similar to that of women ($P = 0.478$). The mean serum Cd in men (0.79 μg/L) was 1.68-fold higher than women ($P = 0.003$), due most likely to smoking, given that serum Cd reflected more recent exposure and that 30% of the men were smokers, while all the women were non-smokers. The mean urine Cd in men (0.48 μg/L) and women (0.39 μg/L) did not differ ($P = 0.719$) although the creatinine-adjusted urine Cd levels tended to be higher in women than men (0.62 vs. 0.54 μg/g creatinine, $P = 0.110$). There were no gender differences in urinary excretion of β2-MG levels. On average, women had 12.4% lower serum zinc and 52.6% lower urine zinc than men, but they