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Restorative Effects of Zinc and Selenium on Cadmium-induced Kidney Oxidative Damage in Rats

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Objective To investigate whether cadmium-induced oxidative stress in the kidney is influenced by zinc and selenium. **Methods** Five groups of rats were maintained: (A) Cd ($CdCl_2$, 400 μg·kg^{-1} day^{-1} intraperitoneal injection); (B) Cd+Zn ($ZnCl_2$, 20mg kg^{-1} day^{-1} hypodermic *injection); (C) Cd+Se (Na_2SeO_3, 350 μg kg^{-1} day^{-1} via a stomach tube); (D) Cd+Zn+Se; (E)* treated with physiological saline as a sham-handled control. The rats were given treatment for a period of 4 weeks. The activities of superoxide dismutase (SOD), glutathione peroxidase (GH-Px), catalase (CAT), and the level of malondialdehyde (MDA) in the kidney tissue were measured to assess the oxidative stress. Urinary lactate dehydrogenase (LDH) activity was used as an indicator of tubular cell damage caused by lipid peroxidation. **Results** In group C and D, activities of SOD (110.5 ± 5.2, 126.8 ± 7.0; $P < 0.05$) and GSH-Px (85.7 ± 4.9, 94.6 ± 7.3; $P < 0.05$) were higher than those in group A(84.7 ± 3.3; 56.9 ± 3.8); and in group B, only the activity of GSH-Px (80.0 ± 4.3, $P < 0.01$) increased in comparison with that in group A (56.9 ± 3.8). Significant increase of MDA ($P < 0.05$) was seen in group B (31.1 ± 4.7) and C (35.0 ± 4.1) when compared with control values (17.2 ± 1.8). No difference was found in the level of MDA between group D (18.9 ± 2.6) and control. The activity of LDH in urine of control group (0.06 ± 0.02) was lower than that of group A (0.46 ± 0.19, $P<0.05$), B (0.10 ± 0.05, $P<0.05$) and C (0.14 ± 0.07, $P<0.05$), and there was no significant change between control (0.06 ± 0.02) and group D (0.08 ± 0.02). **Conclusion** Zinc or selenium could partially alleviate the oxidative stress induced by cadmium in kidney, but administration cadmium in combination with zinc and selenium efficiently protects kidney from cadmium-induced oxidative damage.

Key words:Cd-inducde oxidative stress; SOD; GSH-Px; Zn; Se

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INTRODUCTION

Cadmium and cadmium compounds have a wide variety of industrial applications such as electroplating, pigments, plastics and Cd-Ni batteries. The increasing uses of cadmium and reports of its ubiquitous presence in environment and food materials have caused a great concern over its toxicity. Cadmium typically gives rise to renal damage[1]. After uptake from environment, cadmium is bound to albumin and preferentially taken up by the liver. In the liver, cadmium induces synthesis of metallothionein, and then metallothionein-bound cadmium is transported to the kidney. Because of its low molecular weight, cadmium-metallothionein is efficiently filtered through the glomeruli and thereafter reabsorbed by the tubules[2]. Uptake is probably by pinocytosis and cadmium enters the lysosomes of the tubular cells. In the lysosomes, metallothionein is degraded and "free" cadmium is released to react with sensitive sites in the cell[3]. The kidney damage is usually the tubular type[4].

Free radical-induced lipid peroxidation in biological system is known to produce cellular damage by peroxidising unsaturated lipids. Several lines of evidence indicate that reactive oxygen species are involved in cadmium-mediated tissue damage. For example, acute as well as chronic, cadmium exposure is associated with elevated lipid oxidation rates in the lung, brain, kidney, liver, erythrocytes and testes[5-9]. Cadmium is known to enhance production of free radicals in the kidney and to interfere with the antioxidant defense system[10,11], and Shaikh found that antioxidants such as N-acetyl cysteine, vitamin E, and glycine could depress Cd-induced nephrotoxicity, indicating that oxidative stress plays a critical role in Cd nephrotoxicity[12-14].

At present, there is an increasing interest in the concept that an individual's susceptibility to numerous environmental insults can be influenced by their intake of selected nutrients. One area that has received considerable attention in this regard is that of the influence of one's antioxidant status on their response to environmental oxidative stressors. While there has been a number of studies on the protective effects of antioxidant vitamins and selected phytochemicals, but studies on the ability of essential mineral elements to modulate the effects of environmental toxicants are relatively few[15]. Both zinc and selenium are essential elements, and play an important role in antioxidant defence system. They are also the part of antioxidant enzymes, superoxide dismutase and glutathione peroxidase[16,17]. In order to investigate whether cadmium-induced oxidative stress in the kidney is influenced by zinc and selenium, the activities of superoxide dismutase (SOD), glutathione peroxidase (GH-Px), catalase (CAT), and the level of malondialdehyde (peroxidants, MDA) in the kidney tissue were measured for the assessment of the oxidative stress. Urinary lactate dehydrogenase (LDH) activity was used as an indicator of tubular cell damage due to lipid peroxidation, according to the previous researches[14,18,19].

MATERIALS AND METHODS

Reagents and Chemicals

Cadmium chloride($CdCl_2$), zinc chloride($ZnCl_2$), sodium selenite (Na_2SeO_3, selenium) and most of the chemicals used in this study were purchased from SIGMA Co. (St. Louis, MO, USA).

Animal and Treatment

Male Wistar rats weighing 180-200g were housed separately according to their experimental groups in a room with a 12-h light/dark cycle, and controlled temperature (23-26℃) and humidity (40%-60%). They were randomly divided into five groups (group A-E). Each group was composed of ten animals receiving food and tap water *ad libitum* throughout the experiments, 4 weeks. Cadmium ($CdCl_2$, 400 $\mu g \cdot kg^{-1} \cdot day^{-1}$ intraperitoneal injection, 5 days per week) was administered to the first four groups, so that, one of them, the effect of cadmium alone was evaluated in group A; group B and group C were used to examine possible interactions between cadmium and zinc ($ZnCl_2$, 20mg $\cdot kg^{-1} \cdot day^{-1}$ hypodermic injection, 5 days per week) or selenium (Na_2SeO_3, 350 $\mu g \cdot kg^{-1} \cdot day^{-1}$ via a stomach tube in a volume of 0.5 ml, 5 days per week); group D was treated simultaneously with zinc and selenium. Animals in group E were treated with physiological saline as a sham-handled control.

Body weights were recorded weekly. At the end of experiment, the animals were held in plastic metabolic cages for 24 h, and urine was collected over ice to preserve the activity of lactate dehydrogenase (LDH). No food was given during urine collection but drinking water was provided *ad libitum*. On the 29th day, the rats were sacrificed, and their kidneys were immediately removed and placed on ice.

Preparation of Tissue Extract

Kidney was minced and homogenized in 50 mmol/L of cold sodium phosphate buffer (pH 7.0) containing 0.1 mmol/L EDTA to give 100 g/L homogenate. The homogenates were then centrifuged at 1 000 rpm for 10 min at 4℃. The supernatants were separated and used for enzyme assays and protein determination.

Determinations of SOD, GSH-Px, and CAT Activity in the Kidney

SOD activity was determined at room temperature according to the epinephrine method[20]. This method is based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome. Ten microlitres of tissue extract was added to 970 µl (0.05 mol/L, pH 10.2, 0.1 mmol/L EDTA) of sodium carbonate buffer. 20 µl of 30 mmol/L epinephrine (dissolved in 0.05% acetic acid) was added to the mixture to start the reaction. SOD was measured at 480 nm for 4 min. One unit of SOD was defined as the amount of protein causing 50% inhibition of the autoxidation of adrenaline.

GSH-Px was evaluated by a modified version of Flohe and Gunzler[21]. A mixture consisting of 650 µl (0.05 mol/L, pH 10.2, 0.1 mmol/L EDTA) of sodium phosphate buffer, 100 µl of 0.01 mol/L glutathione (GSH), 100 µl of 1.5 mmol/L NADPH, and 100 µl of glutathione reductase (0.24 units) was added to 50 µl of tissue extract and incubated at 37℃ for 10 min. Following incubation, 50 µl of 12 mmol/L t-butyl hydroperoxide was added to 450 µl of the tissue mixture to start the reaction. GSH-Px was measured at 340 nm for 3 min. The molar extinction coefficient of 6.22 × 103 mol/L cm-1 was used to determine the activity. One unit of activity is equal to the millimoles of NADPH oxidized/ min per mg protein.

CAT activity was assayed as suggested by Aebi[22]. Ten microlitres of ethanol was added to 100 l of tissue homogenate. The tissue mixture was then placed in an ice bath for 30 min and followed by the addition of 10 µl of Triton X-100 RS. 10 µl of the tissue extract was added to a cuvette containing 240 µl (0.05 mol/L, pH 10.2, 0.1 mmol/L EDTA) of

sodium phosphate buffer, and 250 µl of 0.066 mol/L H_2O_2 (dissolved in sodium phosphate buffer) was added to start the reaction. CAT was measured at 240 nm for 1 min. The molar extinction coefficient of 43.6 mol/L cm-1 was used to determine CAT activity. One unit of activity is equal to the millimoles of H_2O_2 degraded/min per mg protein.

Protein content was determined by the method of Lowrys'[23], and bovine serum albumin was used as a reference.

Analysis of the Level of MDA in the Kidney

Lipid peroxidation products were determined by measuring the levels of MDA[24]. To 0.2 ml of homogenate, 0.2 ml of 81 g/L sodium dodecyl sulphate and 1.5 ml of 20% acetic acid were added pH was adjusted to 3.2 with 200 g/L of sodium acetate solution. After the addition of 1.5 ml of thiobarbituric acid, 8 g/L of the mixture was diluted to 4 ml with water and then heated for 60 min in a boiling water bath. It was cooled to room temperature, and 1 ml of water was added and followed by a mixture of n-butanol and pyridine (15:1). The mixture was shaken vigorously and centrifuged at 1 500 × g for 15 min. The absorbance of organic layer was measured at 532 nm and the results were expressed as nmol MDA/g of the kidney.

Determination of Urinary LDH Activity

LDH activity in urine was measured by the method of Hochella and Weinhouse[25].

Analysis of Urinary Creatinine

Creatinine concentration was measured by colorimetry after Jackson[26]. LDH in urine was corrected for creatinine concentration (to be abbreviated as cr).

Data Analysis

Data were calculated separately for each group, and reported as means SD. The significance of the difference between two groups was evaluated by Student's *t*-test. $P<0.05$ was considered to be significant.

RESULTS

Body Weight of Each Group

At the end of experiment, the fourth week, compared with group E, the body weights of group A ($P<0.01$), B ($P<0.05$), and C ($P<0.05$) were lower. No significant difference was found between group E and D (Fig. 1).

Activities of SOD, GSH-Px and CAT in the Kidney

Activities of total SOD, GSH-Px and CAT in the kidneys of control and the other four experimental groups were presented in Table I. As the results shown, the kidney CAT activity was not altered under experimental conditions used in this study. However, significant changes of SOD and GSH-Px were observed. In the kidneys of rats exposed to Cd, the activities of SOD and GSH-Px were lower ($P < 0.05$), whereas the activity of CAT was not changed. In group B, increases ($P < 0.005$) of SOD activity was found. When rats

were simultaneously treated with Cd, Zn and Se, i. e. group D, activities of total SOD and GSH-Px were higher ($P < 0.02$) than those in the kidneys of control animals. In group C and D, activities of SOD ($P < 0.005$) and GSH-Px were higher than those in group A; and in group B, only the activity of GSH-Px ($P < 0.01$) increased in comparison with that in group A.

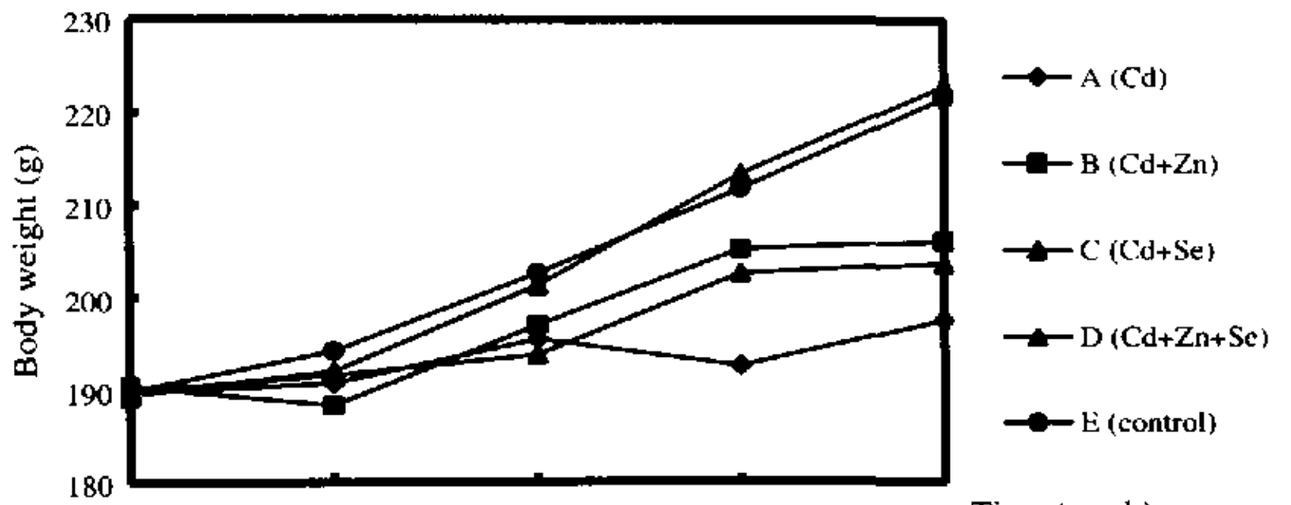

FIG. 1. Changes of body weight in each group.
*Significantly lower than the group E at the same time point ($P < 0.05$).

Level of MDA in the Kidney

Data on MDA in the kidney was shown in Fig. 2. In rats exposed to Cd alone, MDA was higher ($P < 0.01$) with respect to controls. The level of MDA in group B and C was lower than that in group A, but significant increase of MDA ($P < 0.05$) was seen in group B and C when compared with controls. Co-administration to Cd, Zn and Se did not induce significant change in the level of MDA in comparison with control group.

LDH Activity in Urine

The activity of LDH in urine of control group was lower than that of group A ($P<0.05$), B ($P<0.05$) and C ($P<0.05$), and no significant change was found between control and group D. The activity of LDH in group A was higher than that in group B ($P<0.05$), C ($P<0.05$) and D ($P<0.05$, Fig. 3).

TABLE 1

Activities of SOD, GSH-Px and CAT in Kidneys of Each Group ($\bar{x} \pm s$)

Group	n	SOD	GSH-Px	CAT
A (Cd)	10	84.7 ± 3.3**	56.9 ± 3.8**	317.3 ± 20.7
B (Cd+Zn)	10	119.1 ± 6.9**#	80.0 ± 4.3**#	330.7 ± 30.0
C (Cd+Se)	10	110.5 ± 5.2**#△△	85.7 ± 4.9#△	320.2 ± 17.9
D (Cd+Zn+Se)	10	126.8 ± 7.0**#	94.6 ± 7.3#△△	323.8 ± 24.2
E (Control)	10	106.6 ± 2.1	90.6 ± 6.1	338.1 ± 28.0

Note. SOD, units/mg protein; GSH-Px, μmol NADPH oxidized/min per mg protein;
CAT, mmol H_2O_2 degraded/min per mg protein;
v. s. group E, * $P<0.05$; ** $P<0.01$;
v. s. group A, # $P<0.01$;
v. s. group B, △ $P<0.05$; △△ $P<0.01$.

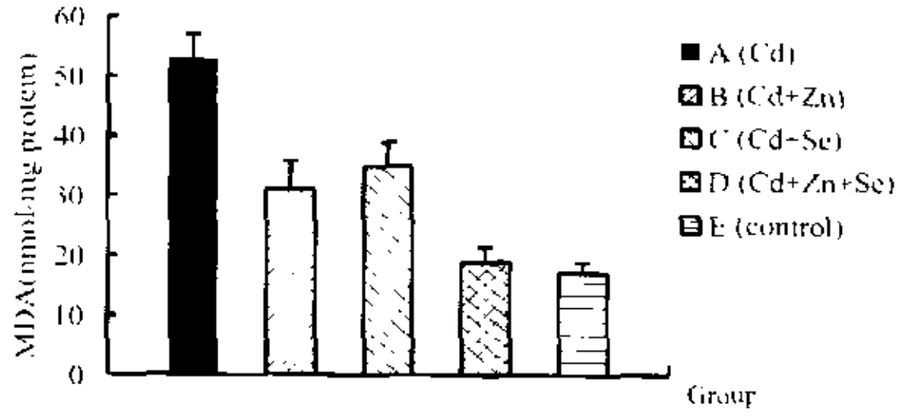

FIG. 2. The level of MDA in the kidneys of each group. compared with control group. $^{*}P < 0.05$, $^{**}P < 0.01$.

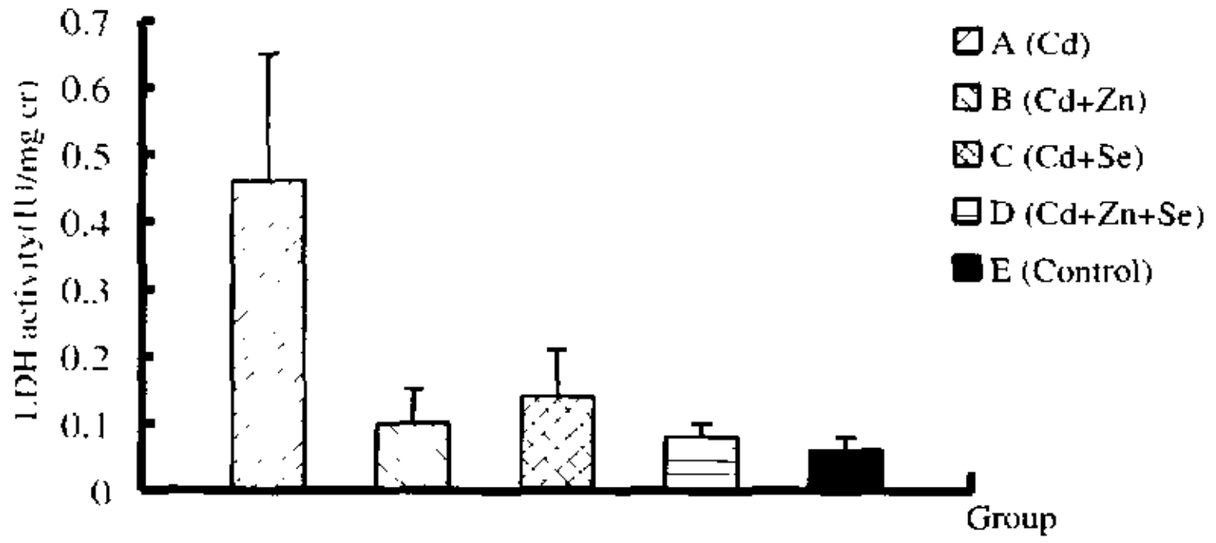

FIG. 3. The activity of urinary LDH in each group compared with control group, $^{*}P < 0.05$.

DISCUSSION

Free radical-induced peroxidative damage to membrane lipids has long been regarded as a critical initiating event leading to cell injury, and this kind of cellular damage occurred in biological system can be prevented by SOD, GSH-Px and CAT, three main enzymes in antioxidant defense system[27]. SOD, GSH-Px and CAT are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying peroxides, and these antioxidant enzymes depend on various essential trace elements for proper molecular structure and enzymatic activity[28].

Previous investigations have shown that Cd inhibits the activity of most antioxidant defense system enzymes[29, 30]. The results from this study also show that activities of SOD and GSH-Px were significantly reduced, whereas activity of CAT was not significantly changed in the kidneys of cadmium treated rats, and the level of MDA in the kidneys and activity of urinary LDH were increased, indicating the occurrence of cell membrane damage in the kidney. Reduction of SOD and GSH-Px was due to the production of free radicals in the kidney enhanced by cadmium. Moreover, SOD is a zinc-dependent enzyme and cadmium has an inhibitory effect on the activity of zinc-containing enzymes[15]. Co-treatment with zinc could alleviate the effect of cadmium on SOD, and the level of renal MDA was significantly lower than cadmium alone, because high supplemental zinc may restore the activity of SOD and suppress the lipid peroxidation[31].

Selenium is a vital trace element that in mammals exerts its most important function

probably via GSH-Px[32]. It has been shown that oral intake of selenium compounds improves antioxidant defense system in the kidney of rats and effectively protects against cadmium induced toxicity[33-35]. Compared with cadmium treated rats, activities of the kidney SOD and GSH-Px were increased in cadmium and selenium co-administrated rats. The level of MDA was decreased, but it was higher than that of the control, and no significant change was found between cadmium-selenium and cadmium-zinc co-treatment group. The possible reason is that SOD is the principle protective enzyme among the enzymatic antioxidant defense systems and is believed to act as a first aspect of antioxidant defence against oxygen free radicals that mediate cytotoxicity or cell death[36], and the activity of SOD induced by zinc is greater than that of selenium. Thus, even activities of SOD and GSH-Px were higher in rats treated in combination with selenium than cadmium alone, but the level of MDA was not lower than that of co-treated group with zinc.

Simultaneously treated with zinc, selenium and cadmium, the activity of SOD in the kidneys was increased, and activities of GSH-Px, CAT, LDH and the level of MDA were not significantly changed compared with control. These findings, especially the observed results of MDA, LDH and body weight, suggest that co-administration of zinc and selenium may prevent the cadmium-induced oxidative impair in the kidney, and the restorative effect of zinc and selenium co-treatment is better than that of zinc or selenium. Among these main enzymes of antioxidant defense system, only CAT remained unaltered, indicating that this enzyme does not play a critical role in preventing cadmium induced oxidative injury[29,37].

From the present results, it can be concluded that the kidney is influenced by cadmium induced oxidative stress, and oxidative impairment may occur in tubular cell of the kidney. Zinc or selenium could partially alleviate the oxidative stress induced by cadmium in the kidney, but cadmium administrated in combination with zinc and selenium can effectively protect the kidney from cadmium-induced oxidative damage.

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