Effects of Long-Term Ingestion of Cadmium-Polluted Rice or Low-Dose Cadmium-Supplemented Diet on the Endogenous Copper and Zinc Balance in Female Rats

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(Received October 2, 2003; Accepted October 8, 2003)

The concentrations of endogenous copper (Cu) and zinc (Zn) in the liver and kidney of female rats were measured after ingestion of cadmium (Cd)-polluted (1.06 ppm) rice or cadmium-supplemented (1.1, 5, 20, and 40 ppm) rice for 12, 18, and 22 months. In the liver, the Cd concentration increases in a dose–dependent manner for the first 18 months. After 18 months, the concentration remained stationary in the low-dose groups, increased in the 5-ppm group, and decreased in the 20- and 40-ppm groups. The Cu concentration was almost unchanged through the experiment, and the Zn concentration increased in a dose–dependent manner. In the kidneys, changes in the Cd concentration resembled that in the liver. The concentrations of Cu increased in a dose–dependent manner at 12 and 18 months. The Zn concentration increased more in the 5-ppm group but not dose dependently.

Key words —— cadmium, zinc, copper, cadmium-polluted rice, rats

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INTRODUCTION

Cadmium (Cd) is a metallic element widely recognized as being toxic to humans and animals which can reach humans through contaminated foodstuffs.[1–3] Epidemiologic surveys have shown that the average Cd intake ranges from 13 to 20 μg/day in the USA and European Union,[4–7] and from 27 to 100 μg/day in Japan.[8,9] In countries where rice is consumed in large quantities, rice becomes a major source of Cd intake. According to the Food Sanitation Law of Japan, the concentration of Cd in rice must not exceed 1 ppm, and if the concentration exceeds 0.4 ppm the rice is considered "semi-polluted" and must not be used for human consumption. Several recent surveys have reported that Japanese rice has the highest Cd concentrations of all Asian countries studied,[9,10] and consequently the daily intake from rice is estimated to be as high as Cd 5.2–29.8 μg per adult.[9]

The results of acute and chronic Cd intoxication of laboratory animals include various degrees of liver and kidney damage. Cd also alters the distribution of several essential elements[11–13] that play very important roles in biological systems.[14] Cadmium accumulation may therefore cause significant changes in the homeostasis of the essential elements, which, in turn, results in several diseases related to either deficiencies or excesses of such elements.

Recently, we have investigated the intestinal absorption of Cd and hepatorenal toxicity in female rats given low amounts of Cd-polluted rice.[15,16] The results showed that the retention rate of Cd did not change with the dosage or the treatment period and that renal toxicity was not induced by long-term oral administration of low amounts of Cd, in contrast to the effects of high-dose Cd administration, although tissue accumulation occurs.

In the present study, the concentration of important endogenous metals, copper (Cu) and zinc (Zn), in the liver and kidneys of rats chronically fed Cd-polluted rice or a low-level Cd-supplemented diet were investigated to establish the effects on these metal balances as a counterpart to the previous absorption and toxicity studies.

MATERIALS AND METHODS

Experimental Design —— A total of 300 female Sprague-Dawley rats, aged 5 weeks, were obtained from Charles-River Japan (Yokohama, Japan).

Table 1. Cadmium Concentration in Diets and their Compositions

Group	Cd concentration in the diets (ppm)	Purified diet (%)	Ordinary rice (%)	Cd-polluted rice (%)[a)]	$CdCl_2$ Supplement (ppm)[b)]
I	0.02	28.0	72.0	—	—
II	1.06	28.0	—	72.0	—
III	1.12	28.0	72.0	—	1.1
IV	4.86	28.0	72.0	—	5
V	20.1	28.0	72.0	—	20
VI	39.5	28.0	72.0	—	40

a) Cadmium concentration in the polluted rice is approximately 1.5 ppm. *b*) $CdCl_2$ supplemented the mixture of purified diet and ordinary rice to obtain the set concentrations of cadmium in the diets.

Six groups of rats, each consisting of 50 animals, were fed diets containing low amounts of Cd chloride or Cd-polluted rice (Table 1). Rats were given diets consisting of 28% purified and 72% ordinary rice (unpolluted or Cd-polluted rice prepared by Oriental Yeast Co. Ltd., Tokyo, Japan). Group I was fed a mixture of purified and ordinary rice and was used as a negative control. Group II was fed a diet of purified rice mixed with Cd-polluted rice with a Cd content of 1.1 ppm to examine the toxic effects of Cd from rice origin. Groups III–VI were fed a mixture of purified and ordinary rice and $CdCl_2$ with Cd contents of 1.1, 5, 20, and 40 ppm. After the commencement of the feeding experiment, the rats were examined daily for clinical signs and weighed once weekly.

The animals in each group were killed at 12, 18, and 22 months (10, 5–7, and all surviving animals, respectively). The rats were deprived of food for 16 hr or more prior to death. The experiment was terminated at month 22 because the total number of surviving animals in the 20-ppm $CdCl_2$-treated group reached the minimum necessary for subsequent analyses of chronic Cd toxicity.

Determination of Cd, Cu, and Zn Levels ——

Analytical Procedure: The samples (0.1–10 g) were weighed into a decomposition vessel, to which 3 ml of HNO_3 was added. Decomposition vessels were soaked in 10% HNO_3 solution for 48 hr and rinsed with water before use. The sample was decomposed in a microwave oven decomposition system under increased pressure. After being cooled to room temperature, the contents of the vessel were placed in a test tube to which water was added to make 10 ml of sample solution. The sample solution was diluted with water to which yttrium and indium solutions were added as internal standards. Cd, Cu, and Zn levels in the sample solution were determined with a indyctively coupled plasma-mass spectrometry (ICP-MS) (HP4500; Hewlett Packard Electric Co., Tokyo, Japan). Calibration curves for the determination of ^{106}Cd, total Cd, Cu, and Zn levels were prepared from the analytical values of the corresponding standard solutions containing internal standard substances. The internal standard method was applied to calculate those levels.

Statistical Analysis: Statistical analyses were performed to evaluate differences between control and Cd-polluted rice or $CdCl_2$-treated animals using the following methods.[17)] Data were analyzed for homogeneity of variance using Bartlett's test.

When the variance was homogeneous among groups, a one-way analysis of variance (ANOVA) was carried out. If significant differences were found using ANOVA, the mean value for each Cd-treated group was compared to that of the controls using Dunnett's test. When the variance was heterogeneous based on Bartlett's test, the Kruskal-Wallis' test was used to check for differences among groups. If significant differences were found, a Dunnet-type rank-sum test was performed. Comparison of different effects was made using Pearson's correlation analysis. The level of significance was set at $p < 0.05$.

RESULTS

Concentration of Cd, Cu, and Zn in the Liver

Cd, Cu, and Zn concentrations in the liver are shown in Table 2. When compared within the same treatment periods, the Cd concentration increased in a dose–dependent manner for the first 18 months of exposure. After 18 months, the concentration remained stationary in the low-dose groups, increased in the 5-ppm group, and decreased in the 20- and 40-ppm groups.

The Cu concentration remained almost unchanged throughout the experimental period (6 to

Table 2. Concentrations of Cd, Cu, and Zn in the Liver of Rats Fed Cd-Polluted Rice or Cd-Supplemented Diet for 12, 18, and 22 months

Group	12 months	18 months	22 months
Cd			
I	nd	0.058 ± 0.035[a)]	0.032 ± 0.027[a)]
II	0.20 ± 0.12	2.8 ± 2.3[a)]	2.7 ± 2.2[a)]
III	1.0 ± 1.4[b)]	2.0 ± 2.1	1.7 ± 1.7
IV	2.0 ± 1.7[b)]	15 ± 12[a,b)]	22 ± 18[a,b)]
V	16 ± 8[b)]	56 ± 33[a,b)]	39 ± 15[a,b)]
VI	32 ± 12[b)]	130 ± 42[a,b)]	85 ± 20[a,b)]
Cu			
I	8.6 ± 2.4	20 ± 21	8.6 ± 2.7
II	8.0 ± 1.8	10 ± 4	9.7 ± 4.1
III	12 ± 8	6.3 ± 1.4	10 ± 6
IV	8.8 ± 2.1	8.6 ± 4.0	7.2 ± 1.7
V	8.4 ± 1.3	9.1 ± 3.3	8.7 ± 4.4
VI	9.1 ± 1.7	9.5 ± 5.2	7.4 ± 1.5
Zn			
I	37 ± 3	37 ± 5	36 ± 6
II	39 ± 4	40 ± 5	38 ± 7
III	42 ± 7	36 ± 7	40 ± 12
IV	42 ± 7	55 ± 16	55 ± 16[b)]
V	53 ± 9[b)]	68 ± 12[b)]	57 ± 13[b)]
VI	60 ± 10[b)]	88 ± 12[a,b)]	71 ± 15[b)]

*nd < 0.01 μg/g. Values of Cd concentration are cited from our previous data.[15)] *a*) Significantly different from to 12 months data, $p < 0.05$. *b*) Significantly different between treatment group and control group (group I), $p < 0.05$.

10 μg/g). The Zn concentration increased in a dose–dependent manner. Correlation coefficients between Cd and Zn are shown in Table 3. Although a correlation between Cd-Cu was not seen ($p > 0.05$, data not shown), a significant correlation coefficient was observed between Cd and Zn after 18 months, except for groups II and VI at 18 months, and groups II and III at 22 months.

Concentration of Cd, Cu, and Zn in the Kidneys

The changes in Cd concentration in the kidneys resembled those in the liver (Table 4). That is, the concentrations increased in a dose–dependent manner for the first 18 months and remained the same thereafter. The concentrations of Cu increased in a dose–dependent manner at 12 and 18 months, and at 22 months the concentrations also increased but not in a statistically significant manner. Although the Zn concentration increased more in the 5-ppm group, the increase was not dose dependent. No correlation between Cd and Cu or Zn in the kidney was observed ($p > 0.05$, data not shown).

Table 3. Pearson's Correlation Coefficients between Cd and Zn Concentrations in the Liver of Rats Fed Cd-Polluted rice or Cd-Supplemented Diet for 12, 18, and 22 months

	12 months	18 months	22 months
I	−0.0189 (10)[a)]	0.798 (7)*	0.767 (11)**
II	0.126 (10)	0.743 (6)	0.609 (9)
III	0.439 (10)	0.912 (7)**	0.202 (9)
IV	0.445 (10)	0.959 (7)**	0.949 (8)**
V	0.0814 (10)	0.964 (5)**	0.918 (6)**
VI	−0.473 (10)	0.0621 (7)	0.812 (9)**

a) Numbers in parentheses are numbers of animals. *$p < 0.05$. **$p < 0.01$.

DISCUSSION

Some studies showed that Cd administered to laboratory animals induced elevated Zn and Cu concentrations in the liver and kidneys. In this study, a significant increase in Zn concentration in the liver and kidneys was observed in all Cd-treated groups. These results were in agreement with our previous results of a 2- and 4-months exposure experiment.[18)]

Table 4. Concentrations of Cd, Cu, and Zn in the Kidneys of Rats Fed Cd-Polluted Rice or Cd-Supplemented Diet for 12, 18, and 22 months

Group	12 months	18 months	22 months
Cd			
I	0.031 ± 0.012	0.20 ± 0.07[a)]	0.12 ± 0.05[a)]
II	1.4 ± 0.3	4.5 ± 2.3[a)]	7.0 ± 4.3[a)]
III	2.8 ± 1.5[b)]	4.8 ± 2.8	5.6 ± 4.2[a)]
IV	7.9 ± 2.4[b)]	20 ± 7[a,b)]	31 ± 16[a,b)]
V	29 ± 4[b)]	67 ± 24[a,b)]	49 ± 16[a,b)]
VI	45 ± 7[b)]	120 ± 20[a,b)]	99 ± 25[a,b)]
Cu			
I	18 ± 7	6.6 ± 2.0[a)]	14 ± 4
II	16 ± 5	9.7 ± 3.0[a)]	16 ± 5
III	15 ± 3	8.0 ± 1.9[a)]	17 ± 9
IV	20 ± 5	9.2 ± 1.4[a)]	17 ± 7
V	24 ± 5	14 ± 4[a,b)]	22 ± 12
VI	29 ± 9[b)]	14 ± 6[a,b)]	29 ± 14
Zn			
I	35 ± 2	29 ± 2[a)]	33 ± 3
II	35 ± 4	33 ± 2	35 ± 4
III	34 ± 3	31 ± 5	37 ± 5
IV	38 ± 2	39 ± 4[b)]	44 ± 6[a,b)]
V	43 ± 4[b)]	40 ± 2[b)]	39 ± 7
VI	44 ± 3[b)]	44 ± 6[b)]	43 ± 7[b)]

Values of Cd concentration are cited from our previous data.[15)] *a*) Significantly different from 12 months, $p < 0.05$. *b*) Significantly different between treatment group and control group (group I), $p < 0.05$.

Cd toxicity affects the intestinal absorption of Zn and Cu because of Cd-induced enteropathy.[19)] We assumed that the enteropathy was not induced based on urinalysis and blood chemistry data and pathologic assessments of the liver and kidneys. Therefore this increase is likely due to the *de novo* synthesis of metallothionein induced by Cd administration.[20,21)] The metallothionein concentration in the kidneys in the 5-, 20-, and 40-ppm groups increased at every time point in a dose–dependent manner.[15)] In the liver, metallothionein increased in the 20- and 40-ppm groups from 12 months, but the liver Cu concentration did not increase. Pearson correlation coefficient analysis also revealed a clear relationship between Cd and Zn, but not between Cd and Cu. Therefore the increase in Zn concentration may not always be based on induction of metallothionein, and we cannot rule out the possibility that the high correlation coefficient between Cd and Zn in the control group had another cause.

Both Cu and Zn are known to be important prosthetic groups for many metalloenzymes, including superoxide dismutase, DNA polymerase, and carbonic anhydrase. Thus any alteration in the homeostasis of these metals can also be detrimental to the activity of these enzymes and may influence human health.

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