CADMIUM ACCUMULATION IN RATS TREATED ORALLY WITH CADMIUM CHLORIDE FOR 8 MONTHS

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ABSTRACT — To investigate the accumulation pattern of cadmium (Cd) in the liver and kidney following Cd intake from diet, female SD rats were fed cadmium chloride (CdCl₂)-containing diets (1.24 and 4.96ppm Cd) for 2 or 4 months. The other rats were fed CdCl₂-containing diets (8, 40, 200, and 600ppm Cd) for 2, 4 or 8 months. The control rats were given diet without Cd addition (lower than 0.01ppm Cd). The concentrations of Cd in the liver and kidney derived from all rats were determined. The concentrations of Cd in the liver and kidney increased depending on the dosage of Cd. The concentrations of Cd in the liver did not reach plateau level even in the 200 and 600ppm groups. On the other hand, the concentrations of Cd in the kidney in the 200 and 600ppm groups reached a plateau level, which was approximately 250 μ g/g. In the 600ppm group, the concentrations of Cd in the kidney reached 250 μ g/g at 2 months, but did not exceed that level at 4 months. In the 200ppm group, the concentrations of Cd in the kidney increased to nearly the level of 250 μ g/g at 8 months. The ratio of the concentrations of Cd in the kidney versus liver decreased as the dosage of Cd increased, suggesting that a low dosage of Cd was distributed preferentially to the kidney, but a high dosage of Cd was distributed to the liver. The relation curves between total amounts of Cd intake and Cd levels in the kidney in the 2-, 4-, and 8-month groups showed a parabola. The curves were shifted in parallel in the direction of higher levels of ingested Cd in order of length of Cd exposure period. These results suggested that when Cd is ingested over a long time at low concentrations, the amount of Cd accumulation in the kidney is small even for equal amounts of total ingested Cd.

KEY WORDS: Cadmium, Rat, Oral, Tissue distribution, Renal toxicity

INTRODUCTION

Cadmium (Cd) has an extremely long biological half-life in the human body and is accumulated in tissues/organs, particularly in the kidney (Friberg *et al.*, 1974; Friberg *et al.* 1986). Because of the high accumulation in the kidney, Cd has been known to be a serious nephrotoxin (Dudley *et al.* 1982; Hoffman *et al.* 1975; Dorian *et al.* 1995) and to produce renal tubular dysfunction characterized by increased excretion of low-molecular-weight proteins, particularly β_2 -microglobulin in humans. With continuous exposure to high

levels of Cd, the effects on the kidney have been reported to become increasingly severe (Friberg *et al.* 1986). So far special attention has been paid as to which level of daily exposure of Cd is safe for human beings (WHO 1989a, b; WHO 1992).

From the human epidemiological data gathered from the literature, it was proposed by the 33rd Joint FAO/WHO Expert Committee on Food Additives (WHO 1989a, b) that the critical concentration of Cd in the renal cortex that produces renal tubular dysfunction in 10% of the population is about 200 μ g/g and the concentration of 200 μ g/g Cd in the kidney could be

used as a starting point for renal effects occurring in an exposed population for the purposes of dose-response calculations of renal toxicity.

Furthermore, the committee pointed out that a regular dietary intake of 175 μg Cd/man/day (2.9 $\mu\text{g}/\text{kg}$ BW/day) would cause the concentration of Cd in the renal cortex to reach a level of 200 $\mu\text{g}/\text{g}$ in 50 years. Based on these calculations, it was concluded that the total intake of Cd should not exceed about 1 $\mu\text{g}/\text{kg}$ BW/day continuously for 50 years, in order that levels of Cd do not exceed 50 $\mu\text{g}/\text{g}$ in the renal cortex, assuming an absorption rate of 5% and a daily excretion of 0.005% of body burden. Therefore, the provisional tolerable weekly intake (PTWI) for Cd was set at 7 $\mu\text{g}/\text{kg}$ BW. These calculations were based on an assumption that the accumulation of Cd is consistently increased when a certain amount of Cd is ingested continuously.

However, it has not been fully elucidated yet as to whether the absorption rate, excretion rate, and critical concentration of Cd in the renal cortex that produces renal dysfunction in the population exposed to a high level of Cd are the same as those in the population exposed to a very low level of Cd. Although Cd was re-evaluated at the 41st meeting of the JECFA (WHO 1993) in 1993, the committee expressed concern about the use of retrospective information for estimating the Cd intake from the dietary intake of rice, and recognized the need to validate the significance of the concentration of Cd in the renal cortex regarded as critical in the general population. For these reasons, the committee maintained the current PTWI of 7 $\mu\text{g}/\text{kg}$ BW, pending future research.

In the present study, we examined the accumulation pattern of Cd in the liver and kidney of female rats given minimum to large amounts of Cd for up to 8 months to clarify the accumulation pattern of Cd in the liver and kidney following Cd intake from diet.

MATERIALS AND METHODS

Animals

The present study was composed of two experiments, i.e., Experiment I and II. Female SD rats aged 4 or 5 weeks obtained from Charles River Japan, Inc. were used in both experiments. Rats were housed individually in stainless steel wire cages in an animal room which was maintained at a room temperature of 21–25°C and a relative humidity of 40–70%, ventilated 16 times an hour. The rats for both experiments were given a sterilized purified diet (lower than 0.01ppm Cd, Oriental Yeast Co., Ltd.) for a 1-week acclimation peri-

od.

Experimental Design

After the acclimation period, the rats for Experiment I, each consisting of 16 rats per group, were given a sterilized purified diet supplemented with CdCl₂ (1.24 and 4.96ppm Cd) for 2 or 4 months. The rats for Experiment II, each consisting of 24 rats per group, were given a sterilized purified diet supplemented with CdCl₂ (8, 40, 200, and 600ppm Cd) for 2, 4, or 8 months. The control rats were given a diet without Cd.

The rats for Experiment I and II were observed for clinical signs once daily, and they were weighed twice weekly. Food intake during the administration was measured twice weekly. The achieved Cd levels (mg/week) were determined based on the food intake and the concentration of Cd in food. Throughout the experiment, rats in all groups were given tap water ad lib. Eight rats from each group in each experiment were killed at each time point (at 2 and 4 months in Experiment I and at 2, 4 and 8 months in Experiment II). Since death was expected to occur before the end of the 8-month administration period in Experiment II, all the animals in the 600ppm group were sacrificed and autopsied at the end of the 4-month administration period.

Estimation of Cd Content in the Liver and Kidney

After exsanguination from the inferior vena cava, the liver and kidney derived from each rat were weighed. Parts of the liver (lateral lobe) and kidney (cortex) were weighed, and then digested with nitric acid in microwave oven (Baldwin *et al.*, 1994). The concentrations of Cd in the digested samples were determined by ICP spectrophotometric analysis.

Statistical analysis

Statistical analyses were made to evaluate differences between the control and Cd-treated groups using the following methods. The numerical data were analyzed for homogeneity of variance using Bartlett's test. When the variance was homogeneous among the 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 control group using Dunnett's test. When the variance was heterogeneous based on Bartlett's test, Kruskal-Wallis's H test was used to check for differences among the groups. If significant differences were found, each Cd-treated group

was compared to the control group using a rank-sum test of the Dunnnett-type. Statistical evaluation was carried out at the 5% level of significance (Dunnnett, 1964).

RESULTS

Clinical signs

During the course of the study in Experiment I, no treatment-related effects were noted in any of the animals.

In Experiment II, hair loss in the thoracic, abdominal, dorsal or femoral region was noted in 7 of 24 rats in the 200ppm group and 11 of 24 rats in the 600ppm group. Emaciation was observed in 7 rats in the 600ppm group. During the administration period, a significantly lower diet intake compared to the control group was noted in the 200 and 600ppm groups. A suppression of body weight gain or weight loss compared to the control group was noted in the 200 and 600ppm groups. The differences attained statistical significance from Day 4. Two rats in the 600ppm group died before

15 weeks of administration due to the exacerbation of clinical conditions.

Pathological findings

In Experiment I, no treatment-related findings were noted in any of the animals. Pathological findings in Experiment II are described in detail in our previous report (Mitsumori *et al.*, 1998).

Cd concentrations in the liver and kidney

In Experiment I, the concentrations of Cd in the liver (Fig. 1a) and kidney (Fig. 1b) in the Cd-treated groups increased depending on the dosage of Cd. In Experiment II, the concentrations of Cd in the liver (Fig. 2a) increased depending on the dosage of Cd; however, they did not reach a plateau level even in the 200 and 600ppm groups. On the other hand, the concentrations of Cd in the kidney (Fig. 2b) reached a plateau level, which was approximately 250 $\mu\text{g/g}$. In the 600ppm group, renal accumulation of Cd reached 250 $\mu\text{g/g}$ at 2 months, but the amount did not exceed

Fig. 1. Time course of cadmium concentration in the liver (a) and kidney (b) of rats in Experiment I. Each point represents the mean $\pm$ SD ($n = 8$). Asterisk (*) indicates a significant difference from the control ($P < 0.05$).

Fig. 2. Time course of cadmium concentration in the liver (a) and kidney (b) of rats in Experiment II. Each point represents the mean $\pm$ SD ($n = 8$). Asterisk (*) indicates a significant difference from the control ($P < 0.05$).

that level at 4 months. In the 200ppm group, the level of Cd reached near 250 $\mu\text{g/g}$ at 8 months.

The ratio of the concentrations of Cd in the kidney versus liver in the 2- and 4-month groups (Fig. 3) decreased as the dosage of Cd increased, and the slope of the correlation curve in the 4-month group was heavier than that in the 2-month group. The relation curves between total amounts of Cd intake and Cd levels in the kidney in the 2-, 4-, and 8-month groups (Fig. 4) showed a parabola. The curves were shifted in parallel in the direction of higher levels of ingested Cd in the order of the length of the Cd exposure period.

DISCUSSION

Several investigators (Decker *et al.* 1957; Miller *et al.* 1968; Moore *et al.* 1973) have studied the cadmium (Cd) absorption from the intestinal tract following oral administration of Cd. However, the effect of the dosage, particularly dosages relevant to human exposure of Cd, on the absorption of Cd has not been examined. Lehman and Klaassen (1986) reported the accumulation of Cd in various organs in rats following a single oral administration at 1 $\mu\text{g/kg}$, which was approximately comparable to the amount ingested by humans from diet. However, humans continuously ingested Cd from diet over a long period of time, and the absorption rate of Cd from the intestinal tract was considered to be varied depending on the exposure period. Therefore, the present study was conducted to assess the Cd absorption rate along with the amounts of Cd accumulated in the liver and kidney by feeding trace amounts of Cd to rats for a long period of time

(maximum 8 months)

In the present study, oral administration of low dosages of Cd resulted in higher concentrations of Cd in kidneys than livers. However, as the dosage of Cd increased, more Cd accumulated in the liver and the ratio of the concentration of Cd in kidney versus liver decreased (Fig. 3). There are some reports (Engstrom and Nordberg, 1979; Lehman and Klaassen, 1986) in which the dosage-dependent distribution of Cd to the kidney and liver following oral administration was investigated, and more Cd was distributed to the kidney at low dosages but the distribution to the liver increased with dosage. It is well known that Cd stimulates a synthesis of the metal-binding protein, metallothionein (MT), in the liver, kidney, and intestine. The MT has a high affinity for Cd and forms the Cd-MT complex. Most of the Cd administered parenterally as an inorganic salt distributes to the liver, but the Cd-MT complex is transported preferentially to the renal tubules by means of glomerular filtration and subsequent tubular reabsorption (Nordberg, 1972; Tanaka *et al.*, 1975; Suzuki and Yamamura, 1979; Cherian and Nordberg, 1983). Therefore, most of the Cd absorbed following oral administration at low dosages of Cd may be bound to MT and distributed to the kidney, whereas at higher dosages, Cd may be absorbed as a free metal and accumulate in the liver. The difference of Cd-accumulation pattern in the kidney or liver depending on the dosages may be related to the forms of Cd, i.e. free or bound to MT, after absorption from the intestine.

The slope of the correlation curve on the ratio of the concentrations of Cd in the kidney versus liver at 4 months was heavier than that at 2 months (Fig. 3). In

Fig. 3. Ratio of the cadmium concentrations in kidney versus liver at 2 and 4 months. Each point represents the mean $\pm$ SD ($n = 8$).

Fig. 4. Relation curves between total amount of cadmium intake and cadmium concentrations in kidney. Each point represents the mean ($n = 8$).

addition, the relation curves between total amounts of Cd intake and renal Cd concentrations of rats given Cd in the diet for 2, 4, or 8 months showed a parabola. They were shifted in parallel in the direction of higher levels of ingested Cd in the order of the length of the Cd exposure period. These results suggested that when Cd is ingested over a long time at low concentrations, the amount of Cd accumulation in the kidney was small even for equal amounts of total ingested Cd. Therefore, when regulating Cd concentration in rice with reference to the results of animal studies, it might be necessary to use the results of animal studies in which low levels of Cd, comparable to the levels ingested by humans, are ingested over a long period of time.

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