Accumulation and Toxicity of Orally Ingested Cadmium in Metallothionein Null Mice

Seiichiro Himeno,* Yukie Yamazaki, and Nobumasa Imura

Department of Public Health and Molecular Toxicology, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, 108–8641, Japan

(Received October 25, 1999; Accepted November 14, 1999)

To investigate the role of metallothionein (MT) on the accumulation and toxicity of orally administered cadmium (Cd), metallothionein null and 129/Sv mice were fed a Cd-containing diet (50 ppm) for four months. Cd concentrations in the liver and kidney of MT null mice did not exceed 10 μg/g throughout the exposure period, while a time-dependent increase in tissue Cd concentrations was observed in the control mice. However, no apparent nephrotoxicity was observed in MT null mice, as is contrary to previous observations in which repeated subcutaneous injections of Cd caused renal dysfunction in MT null mice, though the renal Cd concentrations did not exceed 10 μg/g. The results of this study suggest that the route of Cd administration plays an important role in the manifestation of Cd nephrotoxicity, even in the absence of MT.

Key words —— cadmium, metallothionein, renal dysfunction, oral administration

INTRODUCTION

Cadmium (Cd) is a wide-spread pollutant which accumulates in the kidney, leading to renal dysfunction. Dietary Cd appears to be the major source of Cd uptake in humans, especially in rice-eating populations.[1,2] It has been well documented in animal studies that the route of Cd administration is an important determinant for the subsequent tissue distribution of Cd. Exposure to Cd from the diet results in preferential accumulation of Cd in the kidney, whereas the intravenous injection of Cd results in higher concentrations of Cd in the liver than in the kidney.[3] Metallothionein (MT), a low-molecular-weight metal-binding protein, plays an important role in the tissue distribution of orally administered Cd. Preinduction of MT in the intestine was shown to increase the renal accumulation of Cd.[4] When Cd bound to MT was directly administered to the blood stream of rats, it preferentially accumulated in the kidney.[5,6]

Recently, MT null mice, in which the genes for MT-I and II were disrupted by gene targeting, have been utilized for the elucidation of the role of MT in the metabolism and toxicity of Cd.[7,8] MT null mice exhibited higher sensitivity to Cd toxicity than control mice.[7,8] However, only the lethality or hepatic toxicity of Cd by acute exposure has been investigated in these studies. Recently, Liu *et al.* reported that repeated subcutaneous (s.c.) injections of $CdCl_2$ led to renal damage in MT null mice but not in control mice, while the renal accumulation of Cd in MT null mice was much less than that in control mice.[9] However, it remains unknown whether the fate and toxicity of Cd is affected by the lack of MT when Cd was orally administered to animals long term. The purpose of this study was to examine the accumulation and toxicity of Cd in MT null and control mice receiving Cd from their diet for four months.

MATERIALS AND METHODS

Animals and Diets —— MT null mice and control 129/Sv mice were purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A.) and maintained at Kitasato University. The diet containing 50 ppm of Cd as $CdCl_2$ was purchased from Clea Japan, Inc. (Tokyo). Five-week-old male mice of both strains were fed Cd-containing diets for 0, 1, 2, 3 and 4 months.

Determination of Cd and Metallothionein Concentrations —— The Cd concentration in the liver and kidney was determined by atomic absorption spectrophotometry (SPECTRAA 400, Varian) after acid digestion of tissue samples with HNO_3. MT concentration was determined by a Hg-binding assay described previously[10] with a slight modification. Briefly, $HgCl_2$ instead of [^{203}Hg]-labeled $HgCl_2$ was used for saturating

*To whom correspondence should be addressed: Department of Public Health and Molecular Toxicology, School of Pharmaceutical Sciences, Kitasato University, 5–9–1, Shirokane, Minato-ku, Tokyo 108–8641, Japan. Tel.: +81-3-5791-6266; Fax: +81-3-3442-4146; E-mail: himenos@pharm.kitasato-u.ac.jp.

the metal binding sites of MT, and the Hg concentration in the final supernatant was determined by atomic absorption spectrophotometry using a Mercury Analyzer (RA-2, Nippon Instruments, Tokyo) after acid digestion with HNO_3 at 100℃ for 30 min. MT concentration was expressed as nmol Hg bound to MT.

Assays for Renal Dysfunction —— Urine samples were collected from each mouse placed in a metabolic cage for 4 h on the day of killing. Blood samples were collected from the vena cava under anesthesia. The concentration of blood urea nitrogen (BUN) was measured using the BUN Test Wako. The activities of urinary β-*N*-acetyl glucosaminidase (NAG) and γ-glutamyl transpeptidase (γ-GTP) were measured using NAG Test Shionogi and γ-GTP Test Wako, respectively. Urinary creatinine concentration was determined by Jaffe's method.[11] Histopathological examination of the kidney was carried out after the fixation of tissue samples with neutralized formaldehyde solution, followed by staining with hematoxylin and eosin.

Administration of Radiolabeled Cd —— MT null and 129/Sv mice were orally administered a single dose of [^{109}Cd]-$CdCl_2$ (Amersham Pharmacia Biotech., Tokyo) at a dose of 0.36 μg Cd/kg body weight. Mice were killed at 6, 24, 48 and 72 h after the $CdCl_2$ administration, and the tissue distribution of Cd was determined by the radioactivity of ^{109}Cd using a γ counter (ALOKA Auto Well Gamma System, ARC-300).

RESULTS

As shown in Fig. 1, the time-dependent accumulation of Cd in tissue was different between MT null and control mice. Cd concentrations in both the liver and kidney of the control mice increased with time, whereas those in MT null mice did not exceed 10 μg/g during the whole period of Cd exposure. Hepatic and renal Cd concentrations reached plateau levels at two and one month, respectively, in MT null mice. MT levels in the liver and kidney of MT null mice were under the detection limit, while a time-dependent increase in MT concentration in both tissues was observed in the control mice (data not shown).

To examine whether the lowered Cd accumulation in MT null mice is caused by the reduced

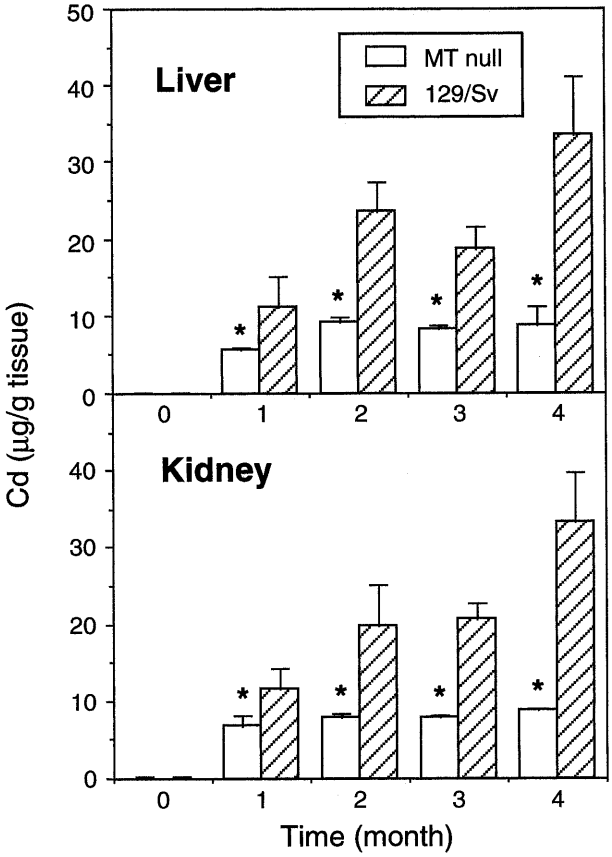

Fig. 1. Time-Dependent Accumulation of Cd in Liver and Kidney of MT Null and 129/Sv Mice Fed Cd-Containing Diet (50 ppm)

*, Significantly different from "129/Sv mice" ($p<0.01$, by t-test).

uptake of Cd from the intestine, a single dose of ^{109}Cd-labeled $CdCl_2$ was orally administered to both strains of mice. As shown in Fig. 2, ^{109}Cd concentrations in the liver and kidney of MT null mice were not less than those in the control mice. In fact, hepatic ^{109}Cd concentrations at 6 and 24 h in MT null mice were higher than those in control mice. Thus, no decrease in intestinal absorption or subsequent tissue distribution of Cd was observed in MT null mice in the short-term experiment.

After four months of dietary exposure to Cd, renal function was examined using biochemical indicators obtained from blood and urine samples. None of the indicators, including BUN, urinary NAG activity or γ-GTP activity, exhibited an increase in either strain of mice (Table 1). In addition, histopathological examination of the kidney did not reveal any lesions in either strain (data not shown).

Table 1. Effects of Dietary Cadmium Exposure on Renal Function in MT Null and 129/Sv Mice

	129/Sv mouse		MT null mouse	
	Time (month)		Time (month)	
	0	4	0	4
BUN (mg/dl)	23.8± 1.2	21.5± 3.1	24.8± 2.9	23.0± 2.5
Urinary NAG (unit/mg creatinine)	104.0±18.4	97.6±26.6	70.4±11.8	91.0± 9.2
Urinary γ-GTP (unit/mg creatinine)	40.7±12.6	43.3± 4.6	66.4±11.7	63.1±50.6

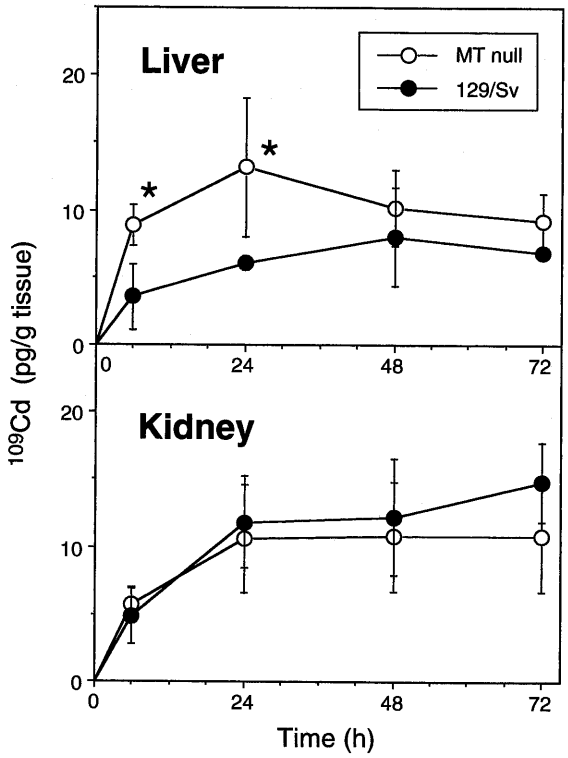

Fig. 2. Tissue Distribution of ^{109}Cd in MT Null and 129/Sv Mice after a Single Oral Dose of [^{109}Cd]-$CdCl_2$
*, Significantly different from "129/Sv mice" ($p<0.05$, by t-test).

DISCUSSION

The results of our present study demonstrated that Cd concentrations in the liver and kidney of MT null mice fed a Cd-containing diet reached a plateau level at one or two months and did not exceed that level thereafter, while the tissue Cd concentrations in control mice increased time-dependently. Restricted Cd accumulation in the kidney was also reported in a study by Liu *et al.*, in which MT null mice received repeated s.c. injections of $CdCl_2$ for 3—10 weeks. In their experiments, renal Cd concentrations did not exceed 10 μg/g even in the group of mice receiving the maximum dose of Cd (0.1 mg/kg/day for ten weeks). Thus, both routes of Cd administration, dietary feeding in the present study and s.c. injections in Liu's study, resulted in the same plateau levels of Cd accumulation in the kidney of MT null mice after long-term exposure. In addition, a short-term experiment with the oral administration of ^{109}Cd (Fig. 2) suggested that the rate of intestinal absorption and subsequent tissue distribution of Cd were almost similar between the two strains. These results suggest that the restricted accumulation of Cd in the kidney of MT null mice is not caused by a factor relevant to the route of Cd administration. Instead, the non-inducibility of MT in the kidney of MT null mice may be responsible for the limited Cd accumulation. In support of this notion, hepatic Cd concentrations also exhibited the same plateau level of 10 μg/g in MT null mice.

On the other hand, no apparent renal dysfunction was observed in MT null mice after four months' exposure to dietary Cd (Table 1). This observation is contrary to the findings by Liu *et al.*[9] They reported that renal damage was observed in MT null mice after repeated s.c. administration of Cd, even though the renal Cd concentration did not exceed 10 μg/g. Although the mechanism of this discrepancy remains unknown, it should be considered that the route of administration and the form of Cd may modulate the renal toxicity of Cd. In the case of s.c. injection, hepatic damage precedes renal damage due to a higher accumulation of Cd in the liver, and the Cd bound to MT released from the liver has been considered to be responsible for the renal toxicity of Cd. However, the study by Liu *et al.* using MT null mice suggested that Cd-MT is not necessarily the cause for Cd nephrotoxicity,[9] although they did not clarify the form of Cd responsible for

its nephrotoxicity. On the other hand, when Cd is orally administered to animals, intestinal proteins or peptides seem to bind to Cd and transport it gradually to the blood circulation. Therefore, the discrepancy in the nephrotoxicity in MT null mice between the two routes of Cd administration might be caused by a difference in non-MT carrier(s) for Cd utilized in each route of administration.

The results of our study and Liu's study demonstrate that both the oral and s.c. administration of Cd results in restricted accumulation of Cd in the tissues of MT null mice similarly, but the manifestation of Cd nephrotoxicity was different between the two routes of administration. Thus, the route of Cd administration plays an important role in the manifestation of Cd nephrotoxicity, even in the absence of MT.

REFERENCES

1) Watanabe T., Koizumi A., Fujita H., Kumai M., Ikeda M., *Tohoku J. Exp. Med.*, **144**, 83—90 (1984).
2) Watanabe T., Shimbo S., Moon C.S., Zhang Z.W., Ikeda M., *Sci. Total Environ.*, **184**, 191—196 (1996).
3) Lehman L.D., Klaassen C.D., *Toxicol. Appl. Pharmacol.*, **84**, 159—167 (1986).
4) Min K.S., Fujita Y., Onosaka S., Tanaka K., *Toxicol. Appl. Pharmacol.*, **109**, 7—16 (1991).
5) Tanaka K., Sueda K., Onosaka S., Okahara K., *Toxicol. Appl. Pharmacol.*, **33**, 258—266 (1975).
6) Cherian G.M., Shaikh Z.A., *Biochem. Biophys. Res. Commun.*, **65**, 863—869 (1975).
7) Michalska A.E., Choo K.H.A., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8088—8092 (1993).
8) Masters B.A., Kelly E.J., Quaife C.J., Brinster R.L., Palmiter R.D., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 584—588 (1994).
9) Liu J., Liu Y., Habeebu S.S., Klaassen C.D., *Toxicol. Sci.*, **46**, 197—203 (1998).
10) Naganuma A., Satoh M., Imura N., *Cancer Res.*, **47**, 983—987 (1987).
11) Bonsnes R.W., Taussky H.H., *J. Biol. Chem.*, **158**, 581—591 (1945).