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Time-dependent Changes of Cadmium and Metallothionein after Short-term Exposure to Cadmium in Rats

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The time-dependent changes in cadmium (Cd) concentration were studied in Female Sprague-Dawley (SD) rats during and after Cd exposure via drinking water (10 and 50 ppm) for 30 days. The cadmium concentration in muscle, liver, kidney, blood plasma, and urine, and the metallothionein concentration in blood plasma were determined every 10 days during exposure and every 7 days after exposure for 3 weeks. The muscle Cd concentration did not change during, and neither after, exposure. The liver Cd concentration increased from 1.4 to 3.3 (at 10 ppm) and from 6.1 to 10.1 folds (at 50 ppm) during exposure and remained higher than those of controls in both groups even during post-exposure period. The kidney Cd concentrations were 2.3 to 5.1 (at 10 ppm) and 4.9-14.0 folds (at 50 ppm) higher than those of controls during exposure and also remained elevated during the post-exposure period. Plasma Cd concentrations were not significantly different from those of controls in both groups. Urine Cd concentrations were more than 2 folds (at 10 ppm) and 6.5 to 12.6 folds (at 50 ppm) higher than those of controls but rapidly decreased over the 7 days of withdrawal. Blood plasma metallothionein concentrations were more than 2.4 folds (at 10 ppm) and 3.1 to 7.4 folds (at 50 ppm), and they remained elevated till 7 days (10 ppm) and 14 days (at 50 ppm) after exposure. Our data support that Cd in urine could be a useful biomarker during Cd exposure period and metallothionein in blood plasma could be as a supportive biological marker for during and post Cd exposure.

Key words: Cadmium, Biomarker, Short-term exposure, Metallothionein, Rat

INTRODUCTION

Cadmium (Cd) is an industrial and environmental pollutant, and toxic to several organs, mostly causing hepatotoxicity on acute poisoning and nephrotoxicity following chronic exposure. Although Cd is present at low concentration in soil, it's accumulation in soil and hence through the dietary intake may cause to health risk in human and animal. Cadmium also seriously affects enzyme activities involved in oxidative stress and apotosis and it interferes especially with antioxidant enzyme (Casalino *et al.*, 2002; Hussain *et al.*, 1987; Lopez *et al.*, 2003). According to recent reports, chronic Cd exposures produce reduction in sperm motility (Benoff *et al.*, 2008), increase the risk of cancer and cardiovascular disease in man (Menke *et al.*, 2009), and Cd levels in blood is associated with a modest elevation in blood pressure (Tellez-Plaza *et al.*, 2008).

Cadmium readily binds to and induces the production of metallothionein (MT), a cysteine-rich, metal binding protein. MT-bound Cd does not easily uptake and is responsible for retention of Cd within cells and its' long half life about 10-30 years in human (Gnoick, 2008). Within hepatocytes, MT binds to Cd and decrease its' hepatotoxicity. But the Cd-MT complex is nephrotoxic in the kidney and it may play a role in chronic poisoning in humans (Hooser, 2007). Metallothionein is widely recognized as an important protein of a cells defense mechanism against and recovery from environmental heavy metal insult. It can bind to metal ions and needed for normal metabolism (Zn, Cu,) and heavy metal pollutants (Cd, Pb, Hg). It can be induced by metal ions as well as cytokines, hormones, cytotoxic agents, organic chemicals, and stress (Haq *et al.*, 2003; Klaassen *et al.*, 1999).

Biomarkers are useful roles in the toxicological and biological evaluation of the human and animal exposure to

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environmental chemicals such as heavy metals and organic compound. Generally, Cd concentration in urine could be regarded as an indicator of body burden of Cd, while Cd concentration in blood may reflect ongoing exposure in biological monitoring. For example, MT levels in great tits and toadfish can be applied to monitor the response to heavy metal pollution (Campana *et al*., 2003; Tellez-Plaza *et al*., 2008). Today, β2-microglobulin is the most extensively used biomarker of chronic Cd exposure, which is induced by renal tubular damage. However, it is very easily degraded at a urinary pH below 5.5 and may therefore be an uncertain biomarker while it is often used as general maker of renal failure during chronic diseases (Nordberg and Nordberg, 2000). Therefore, urinary metallothionein should be a more sensitive biomarker of renal dysfunction than β_2-microglobulin, which is the most commonly used as indicator for renal toxicity. Induced MT mRNA expression of peripheral blood lymphocytes in Cd exposed populations may reflect to the ability of tissue to synthesis MT (Chen *et al*., 2006). Metallothionein induction in human hepaG2 cell could be used as useful tools for monitoring complex metal mixture in aquatic sediments (Shea *et al*., 2008). Both animal and epidemiological studies indicated that MT mRNA levels were related to Cd exposure (Cosma *et al*., 1991; Yamada and Koizumi, 2001). Metallothionein in rat kidney was the most sensitive biomarker among metallothionein content, catalase, GSH-Px, caspase 3, ALT, AST activities to subchronic exposure to organic and inorganic Cd (Hispard *et al*., 2008).

Although much attention has been focused on Cd distribution in tissue or on Cd-induced toxicity, there is still a lack of information regarding the time-dependent changes of Cd in major tissue and biomarkers in biological samples after short-term Cd exposure in laboratory animal. The aim of the present study is to investigate the changes of Cd concentration in tissue as well as metallothionein concentration in blood plasma after short-term Cd administration to rats.

MATERIALS AND METHODS

Chemical and reagents. Reagent grade cadmium chloride, metallothionein, ethyl acetone, propanol were purchased from Sigma-Aldrich (St. Louis, USA).

Animal study design. Fifty-four 6 weeks old female Crj:CD (SD) rats were provided by National Veterinary Research and Quarantine Service (Anyang, Korea). Rats were provided with tap water and a commercial diet ad libitum. The animal room was maintained at a temperature of 24 ± 2°C, a relative humidity of 50 ± 20%, and a 12 h light/dark cycle. All animals were cared for according to the Code of Laboratory Animal Welfare and Ethics of the NVRQS. Experimental design was approved by the NVRQS Animal Welfare Committee. Rats were divided into 3 groups of 18 rats each. Groups were maintained on a commercial diet and administered distilled water for 30 days. Distilled water contained 0ppm (control), 10 ppm (low-dose) and 50 ppm (high-dose) Cd. We conformed that CdCl2 was fully dissolved in water at high dose. Three rats were euthanized every 10 days during the exposure period and every 7 days thereafter for 21 days. Rats were fasted for 18 hours before euthanasia, and then exsanguinated via incision of the abdominal aorta under anesthesia. Muscle, kidney, liver, blood and urine were obtained from euthanized rats and placed in conical plastic tubes guaranteed free of Cd by the manufacturer (Corning Inc).

Determination of cadmium content in muscle, kidney and liver. The amount of Cd was determined with graphite furnace atomic absorption spectrophotometry (GF-AAS). One gram of each tissue was placed in a high form of porcelain crucible and heated at 90~95°C for 10 min. Furnace temperature was slowly increased from room temperature to 500°C for 1 hour. Samples were ashed overnight until white or grey ash residue was obtained. 5 m*l* of 25% nitric acid, ash was dried on a 90~95°C hot plate. Samples were again ashed at 500°C for 1 hour, 5 m*l* of 25% nitric acid was added to dissolve the residue on a 90~95°C hot plate. Dissolved samples were placed in Cd free centrifuge tubes and distilled water was added to a volume of 25 m*l*. Each sample was centrifuged at 2,000 ×g for 10 min. Supernatant was used for the determination of Cd using an atomic absorption spectrometer (PerkinElmer AAnalyst 600) equipped with graphite furnace and autosampler (PerkinElmer AS 800) with the method recommend by supplier. A standard curve was generated for each run.

Analysis condition followings; wavelength (283.3 nm), Argon flow (250 m*l*/min), injection volume (20 μ*l*), slit width (0.7 nm), drying step 1 (110°C, 1 sec ramp and 30 sec hold), drying step 2 (130°C, 15 sec ramp and 30 sec hold), ashing (850°C, 10 sec ramp and 20 sec hold), atomization (1,600°C, 5 sec), cleaning (2,450°C, 1 sec ramp and 3 sec hold), modifier (5 μ*l* of 50 μg $NH_4H_2PO_4$ with 3 μg $Mg(NO_3)_2$.

Determination of metallothionein concentration in blood plasma. Metallothionein was derivatized and analyzed according to the modified method (Miyari *et al*., 1998). Blood plasma was boiled for 5 min and then took 50 μ*l* of plasma into ependorf tube and added 1.75 m*l* of acethylacetone. After centrifugation at 2,000 ×g for 10 min at 4°C, took 150 μ*l* of supernatant and added 350 μ*l* of 1 M borate buffer with 5 mM EDTA (pH 10.5), 10 μ*l* of tirbutyl phosphine (TBP) in isopropanol and 40 μ*l* of ammonium 7-fluorobenz-2-oxa-1,3-diazole-4-sulfonate (SBD-F) in water. After heating the mixture at 60°C for 30 min, 50 μ*l* of 4 N HCl was added, and then filtered with disposable filter (25 mm, 0.22 μm nylon) into amber vial. The metallothion-

ein concentration was determined by HPLC/fluorescence detection (Waters, USA). Mobile phase consisted of 20 mM pottasium phosphate buffer (pH 7.5)/acetonitrile/methanol (80 : 18 : 2) with flow rate, 0.75 m*l*/min and injection volume, 20 μ*l*. A ODS C_{18}, (250 mm × 3.9 mm, 5 μm particle size) column was used and fluorescence detector was set at excitation 384 nm/emission 510 nm.

Statistical analysis. The concentrations of Cd and MT were analyzed by break down & one-way ANOVA, followed by Duncan test as post-hoc comparison using Statistica program (Ver. 5.5).

RESULTS

Cadmium accumulation in muscle, liver and kidney. Rats consumed about 20 m*l*/day/rat of $CdCl_2$ solution during exposure but we did not observe any significant difference in water consumption between treatment groups and vehicle control (Data was not shown). Cd amount in muscle did not change significantly neither during exposure nor post exposure in the low and high Cd exposure groups (Table 1). The liver Cd amounts were 1.4 to 3.3 folds higher than those of control rats and it remained 1.4 to 2.3 folds higher after exposure ended in the low exposure group. In the high-dose exposure group, Cd amounts were increased time-dependently (6.1 to 10.foldsfolds higher to controls) during exposure and still remained 3.7~5.0 folds higher post exposure (Table 2). Renal Cd concentrations were 2.3 to 5.1 folds higher in the low exposure group compared to the control group and remained 2.1 to 4.1 folds higher post exposure period. In high-dose group, the kidney Cd concentrations increased time dependently to 4.9 to 14.0 folds higher than control concentration and remained elevated after exposure till 3 weeks. The contents of Cd in kidney were 2.1 to 3.3 folds higher in high exposure group compared to those of low exposure group during and throughout the post exposure period (Table 3, Fig. 3).

Cd concentration in blood plasma and urine. Blood plasma Cd concentrations were no significantly difference compared to the control group in the both low and high exposure groups (Fig. 1, Fig 3). Urine Cd concentrations were more than 2 folds higher in low exposure group to controls, but rapidly decreased over the next 7 days. Urine Cd concentrations were 6.5 to 12.6 folds higher in the high exposure group compared to controls and decreased to control level after stop of exposure (Fig. 1, Fig. 3).

Metallothionein concentration in blood plasma. The MT concentrations in plasma were more than 2.4 folds higher in the low exposure group to controls and remained

Table 1. Change of cadmium concentration in muscle tissue during and after administration of $CdCl_2$ via drinking water (μg/mg)

Groups	Administration period (days)			After administration (days)		
	10	20	30	7	14	21
D.W (n = 3)	51 ± 25	65 ± 10	61 ± 11	79 ± 5	50 ± 11	53 ± 8
Low Cd (10 ppm, n = 3)	63 ± 17	94 ± 14	57 ± 13	90 ± 8	67 ± 2	60 ± 23
High Cd (50 ppm, n = 3)	92 ± 13	86 ± 17	83 ± 14	80 ± 2	63 ± 25	45 ± 8

Table 2. Change of cadmium concentration in liver during and after administration of $CdCl_2$ via drinking water (μg/mg)

Groups	Administration period (days)			After administration (days)		
	10	20	30	7	14	21
D.W (n = 3)	144 ± 13	202 ± 25	218 ± 62	497 ± 29	257 ± 25	305 ± 23
Low Cd (10 ppm, n = 3)	205 ± 29	**660 ± 91	**666 ± 84	**901 ± 82	**585 ± 38	*440 ± 20
High Cd (50 ppm, n = 3)	**873 ± 55	**1,620 ± 280	**2,199 ± 18	**1,856 ± 119	**1,200 ± 87	**1,524 ± 87

*Significantly different from vehicle at $p < 0.05$.
**Significantly different from vehicle at $p < 0.01$.

Table 3. Change of cadmium concentration in kidney during and after administration of $CdCl_2$ via drinking water (μg/mg)

Groups	Administration period (days)			After administration (days)		
	10	20	30	7	14	21
D.W (n = 3)	236 ± 17	208 ± 52	193 ± 31	377 ± 15	487 ± 20	495 ± 26
Low Cd (10 ppm, n = 3)	**537 ± 53	*737 ± 45	**990 ± 35	**1,542 ± 106	**1,042 ± 71	**1,027 ± 95
High Cd (50 ppm, n = 3)	**1,161 ± 131	**2,434 ± 846	**2,699 ± 95	**4,165 ± 332	**2,603 ± 89	**2,043 ± 124

*Significantly different from vehicle at $p < 0.05$.
** Significantly different from vehicle at $p < 0.01$.

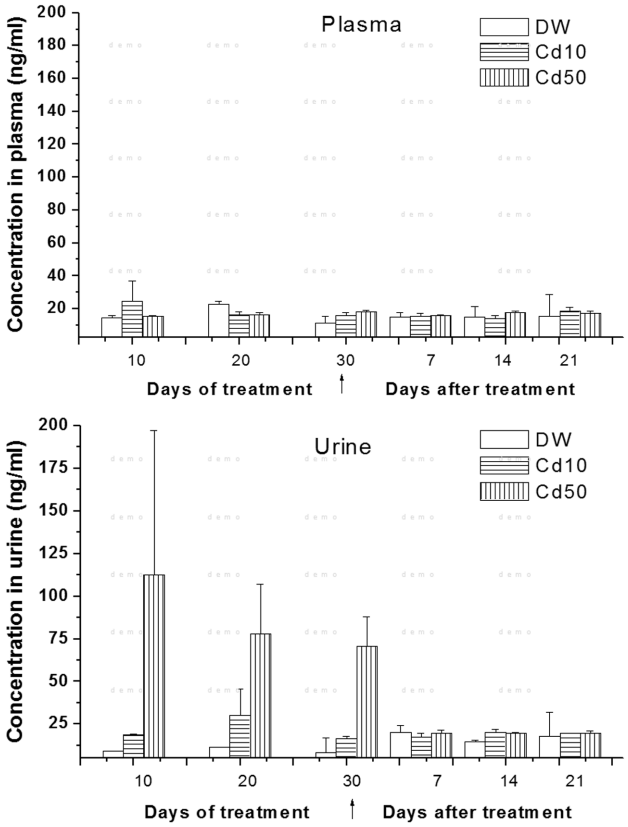

Fig. 1. Changes of cadmium concentrations in rat blood plasma and urine after the administration of DW and cadmium (10, 50 ppm), respectively, in rats. Values are mean ± SD. One urine sample was collected from DW treatment group at 10 and 20 days, Cd 10ppm treatment group at 3 weeks after treatment.

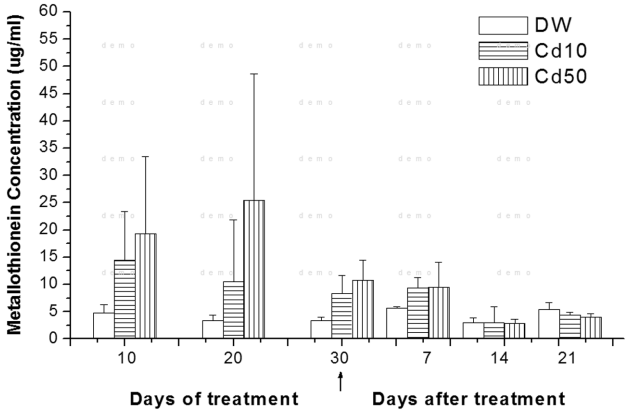

Fig. 2. Change of metallothionein concentration in rat blood plasma after the administration with DW, Cd 10 ppm and Cd 50 ppm for 30 days, respectively. Values are the mean ± SD.

elevated till 7 days after exposure. In high exposure group, plasma MT concentrations were increased 3.1 to 7.4 folds compared to controls during exposure and decreased time-dependently to those of control at 14 days after exposure

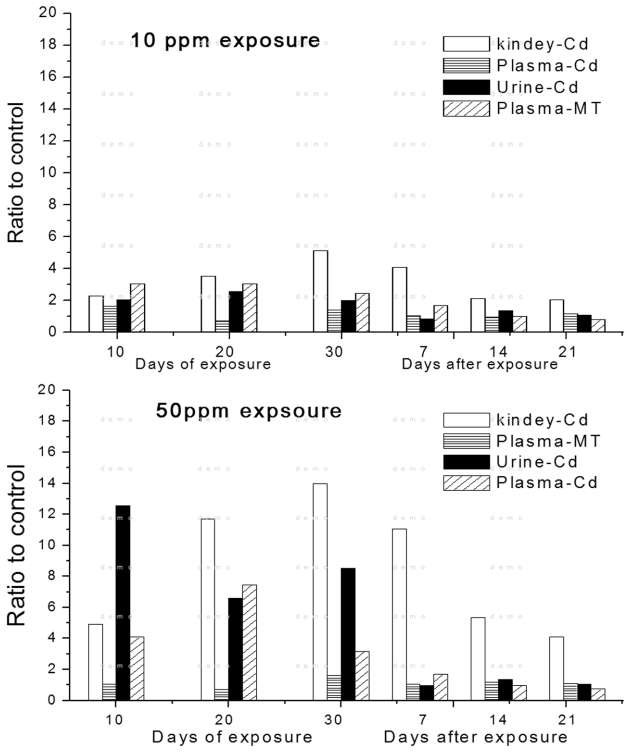

Fig. 3. Comparison of biomarkers for cadmium during and after exposure. Data are represented as mean ratio to control each time period.

(Fig. 2, Fig. 3).

DISCUSSION

Cadmium did not accumulate in skeletal muscle of rats in this experiment. This supports previous studies showing that muscle is not a primary target organ for Cd in laboratory animals. We could not find Cd residue data in muscle of rats exposed to Cd by orally but in domestic mammals such as cattle and sheep, only a small amount of Cd was found in muscle after high amount of Cd exposure (Puls, 1994). When Cd is absorbed over the intestinal mucosa it enters into the liver via portal and lymph systems, and binds to metal binding protein such as metallothionein, which alter its' toxic activity. The ability of liver to sequester Cd is attributable to binding by metallothionein produced in liver, but higher Cd/metallothionein ratios are seen with increasing Cd concentrations as a result of saturation of binding (Sugawara and Sugawara, 1991).

In our study the concentration of Cd in liver was higher in both experimental groups compared to the control group during the exposure period, but decreased rapidly once exposure ended in the high-dose rats. In high-dose rats, hepatic Cd concentrations were still remained high levels during the post exposure period, suggesting that Cd have

high affinity to hepatic metallothionein very stably in liver and then redistribute to other target organs after exposure. Free circulating Cd can bind to renal metallothionein in the kidney and thereby accumulate in renal cortex. In this experiment, renal Cd concentrations increased in a dose and time-dependent manner during exposure, and they were maintained elevated concentration till 21 days after exposure in the both groups. This may have been related to the stable interaction between metallothionein and Cd, by which Cd continues to accumulate in the kidney during post exposure period. At steady state the kidney and liver have the highest concentrations of Cd and contain about 30 and 20% of the body burden of Cd, respectively (Bernard and Lauwerys, 1986; Friberg and Elinder, 1983; Goyer, 2008). In this experiment, the accumulation and depletion pattern of renal Cd showed similar pattern with those of hepatic Cd for both treatment groups throughout the experiment. As expected, kidney and liver were major target sites of Cd.

When studying of the health effects of environmental contaminant, it is critical to develop biomarkers that measure exposure or biological response. For cadmium, blood Cd concentrations indicate circulating Cd and thus is a proxy for recent acute Cd exposure as well as release of Cd from tissue stores. The concentration of blood Cd is the most widely used biomarker of exposure, largely because it is the easiest to obtain and because absorbed Cd is transported in the blood, bound mainly in red cells or bound to high molecular weight proteins in the plasma (Bernard and Lauwerys, 1986; Goyer, 2008). Since blood concentrations are strongly influenced by recent exposure they may not reflect long-term exposure, particularly to intermittent or infrequent doses. Furthermore blood Cd alone does not indicate the toxic effect of Cd because of individual differences in the degree of tolerance to Cd. Since the free plasma Cd is more bioavailable than the Cd bound to RBC or proteins, plasma Cd is likely to be a better indicator of toxic effects. Because of the pharmacokinetics of Cd metabolism and clearance, the urine Cd concentration changes more rapidly and may vary independently of the blood Cd concentration. Till now, urine Cd concentrations have not been as thoroughly validated as blood Cd concentrations as a biomarker of external exposure or as a predictor of health effects. In the present study, blood plasma Cd concentrations were not significantly increased in both treatment groups and we hypothesize that it is due to the fact that most of Cd may be bound to RBC which is supported by other report (Bernard and Lauwerys, 1986; Goyer, 2008) In the present study we, however, did not determine whole blood Cd concentrations therefore a follow up study it is required in order to identify more exact mechanism.

In our study, urine Cd concentrations were significantly increased in a dose dependent manner, but rapidly decreased over the following post exposure 7 days period to finally reaching normal values. This result shows that urine Cd could be the most useful biomarker for short-term Cd exposure in rats. In acute exposure to Cd, most of the Cd is distributed to the liver while redistribution to the kidneys occurs following hepatic production of metallothionein. The binding of Cd to metallothionein prevents the free Cd ions from exerting their toxic effects. Free Cd ions in the cells as a result of the degradation of metallothionein initiate the synthesis of new metallothionein which then binds the Cd and thereby protect the cell from the highly toxic free Cd ions (WHO, 2001). In this experiment, Cd administration did not affect on the feed consumption or bodyweight gain (data were not presented). So, we hypothesize that Cd strongly binds so hepatic and renal metallothionein, which subsequently decrease urine Cd concentration after termination of exposure.

About 80~90% of Cd in the body is bound to metallothionein (Friberg *et al*., 1979). Even though acute exposure of experimental animals to a high dose of $CdCl_2$ has little nephrotoxic effect and nephrotoxicity with either repeated parental small daily doses of $CdCl_2$ or following prolonged oral ingestion of this substance may stimulate the synthesis of a cystein-rich metal-binding protein, metallothionein (MT), in the liver, to which Cd binds and forms a CdMT complex (6~7 kDa) that may be released into circulation (Dorian *et al*., 1995). The circulating CdMT may be filtered by the glomeruli, endocytosed in proximal tubule cells, and degraded in lysosomes and the released Cd may then stimulate production of MT in proximal tubule cells (Dorian *et al*., 1992; Dudley *et al*., 1985). Field study in fish showed that MT levels, especially in the gills, the first target tissue of these contaminants, can be used as biomarker of exposure to Cd (Bebianno *et al*., 2004; Cheng *et al*., 2009). In this experiment, MT concentrations in plasma were more than 2.4 folds higher in the low-dose group and remained elevated till 7 days after exposure period. In high-dose group, plasma MT concentrations were increased 3.1 to 7.4 folds during exposure and decreased time-dependently but decreased to those of control at 14 days after exposure period while urine Cd concentrations were rapidly decreased over the next 7 days to normal value. This suggest that Cd accumulated and remained in kidney without excreting in urine after post exposure but some liver originated Cd still stimulated the expression of metallothionein even after exposure. In summary Cd in urine was the most useful biomarker for Cd during exposure and MT in plasma could be used as a supportive biological marker for even though post Cd exposure.

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