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

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(Received January 27, 2010; Revised May 1, 2010; Accepted May 5, 2010)

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, its 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 apoptosis 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 (Gonick, 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
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 β2-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 sub-chronic exposure to organic and inorganic Cd (Hispard et al., 2006).

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 C57CD (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 0 ppm (control), 10 ppm (low-dose) and 50 ppm (high-dose) Cd. We confirmed 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 ml 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 ml 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 ml. 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 A Analyst 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 ml/min), injection volume (20 μl), slit width (0.7 nm), drying step (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 μl of 50 μg NH4H2PO4 with 3 μg Mg(NO3)2).

**Determination of metallothionein concentration in blood plasma.** Metallothionein was derivatized and analyzed according to the modified method (Miyairi et al., 1998). Blood plasma was boiled for 5 min and then took 50 μl of plasma into ependorf tube and added 1.75 ml of acetylaceetone. After centrifugation at 2,000 ×g for 10 min at 4°C, took 150 μl of supernatant and added 350 μl of 1 M borate buffer with 5 mM EDTA (pH 10.5), 10 μl of tiritubyl phosphine (TBP) in isopropanol and 40 μl 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 μl of 4 N HCl was added, and then filtered with disposable filter (25 mm, 0.22 μm nylon) into amber vial. The metallothionein-
tein concentration was determined by HPLC/fluorescence detection (Waters, USA). Mobile phase consisted of 20 mM potassium phosphate buffer (pH 7.5)/acetonitrile/methanol (80 : 18 : 2) with flow rate, 0.75 ml/min and injection volume, 20 µl. A ODS C₁₈, (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 ml/day/rat of CdCl₂ 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 folds 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 CdCl₂ 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 |

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

### Table 2. Change of cadmium concentration in liver during and after administration of CdCl₂ 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** | **1,042 ± 71** | **1,027 ± 95** |

*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 CdCl₂ 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.
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. 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 expo-
sure 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 Lauw-
erys, 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 mea-
sure 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 trans-
ported 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 differ-
ces 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 metab-
olism 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 concentra-
tions 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 expo-
sure in rats. In acute exposure to Cd, most of the Cd is dis-
tributed 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 termina-
tion of exposure.

About 80–90% of Cd in the body is bound to metallothio-
nein (Friberg et al., 1979). Even though acute exposure of
experimental animals to a high dose of CdCl₂ has little
nephrotoxic effect and nephrotoxicity with either repeated
parental small daily doses of CdCl₂ 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 fil-
tered 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-depen-
dently but decreased to those of control at 14 days after
exposure period while urine Cd concentrations were rap-
idly decreased over the next 7 days to normal value. This
suggest that Cd accumulated and remained in kidney with-
out excreting in urine after post exposure but some liver
originated Cd still stimulated the expression of metallothio-
nein 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.

**ACKNOWLEDGEMENTS**

This project was supported by research funds from
National Veterinary Research and Quarantine Service,
Republic of Korea. The authors also express deep thanks to
Dr Bischoff at Cornell University for her kind review on
this paper.

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