Changes in Tissue Metals After Cadmium Intoxication and Intervention With Chlorpromazine in Male Rats [1]

YANG XIAO-FANG [2], WANG SHU-YI, ZHAO REN-CHENG, AO SHU-QING, XU LI-CHUN, AND WANG XIN-RU

Institute of Applied Toxicology, Nanjing Medical University, Nanjing 210029, China

Cadmium (Cd), one of the most dangerous heavy metals, has a very similar ionic radius to calcium (Ca). The interference of cadmium in calcium homeostasis may play an important role in cadmium toxicity. Recent reports indicate that calmodulin (CaM) inhibitors such as trifluoperazine and chlorpromazine (CPZ) could protect rodents against cadmium toxicity. It was also reported that pretreatment of mice with zinc (Zn) could reduce the adverse effects induced by cadmium. The aim of this study is to determine whether Cd changes the balance of other essential metals such as Zn and copper (Cu) in rat tissues, and whether CPZ can reverse these changes which are induced by cadmium intoxication. Adult male Sprague-Dawley (SD) rats were injected intraperitoneally (ip) with cadmium chloride ($CdCl_2$) (0.2, 0.4, 0.8 mg Cd/kg body weight) alone and 0.4mg Cd/kg in association with CPZ (5 mg/kg) daily for a week. The control animals were injected with normal saline only. The results showed that the cadmium content in the liver, kidney and testis increased significantly with a dose-response relationship. Cadmium treatment markedly increased the Zn and Ca content in some of the tissues. Hepatic and renal metallothionein (MT) increased significantly after cadmium intoxication. CPZ treatment, however, reduced cadmium content in liver, but not blood and kidney. CPZ seemed to decrease the content of MT in liver and significantly increase the amounts of MT in kidney. These data suggest that the intervention of cadmium with tissue essential metals may play a role in cadmium toxicity in rats, and calmodulin inhibitors to some extent can reduce the adverse effect of cadmium by decreasing the cadmium load in tissues and reversing the unbalance of essential metals.

INTRODUCTION

Cadmium, a ubiquitous heavy metal, is known to cause adverse effects on both human and non-human. The mechanism of Cd toxicity is presumed to involve cross reaction between cadmium and calcium with calmodulin dependent systems because of their similar ionic radius (Sutoo, Akiyama, and Imamiya 1990). Recent reports indicate that calmodulin antagonists such as CPZ and trifluoperazine might protect rodents against cadmium toxicity (Shiraishi and Rehm 1994; Robert, Niewenhuis, and Prozialeck 1987; Yang and Yang, 1997). In addition, metallothionein, a metal-binding protein, is considered to be related to the absorption and distribution of cadmium. It has also been reported that MT might play a role in cadmium tolerance. Some essential metal ions (such as Zn^{2+}, Cu^{2+}) and some heavy metal ions (such as Cd^{2+}, Pb^{2+}) can induce the synthesis of MT in tissues. The essential metal ions also play an important role in the activation of some key enzymes in tissues. Therefore, we presumed that the loss of metal balance in tissues might be a possible

[1] This project was granted by National Natural Science Foundation of China (No: 39640006).

[2] Corresponding author: Yang Xiao-fang. E-mail: xfyang@njmu.edu.cn

0895-3988/2000
CN 11-2914

mechanism of Cd toxicity. It is interesting that treatment with calmodulin inhibitors can increase the MT gene expression in rats (Shiraishi and Rehm, 1994). In a previous study, we also found that 5mg/kg CPZ had protective effects against cadmium induced renal toxicity in rats, which might be due to an increased excretion of cadmium from urine (Yang and Yang, 1997). In this study, we investigated if the concentration of tissue metals such as Zn, Cu and Ca was affected by cadmium intoxication and if CPZ modulates such changes, which may provide additional evidence for development of a hypothesis for cadmium toxicity in rats.

MATERIALS AND METHODS

Chemicals

$CdCl_2$ is a product of Tianjin Chemical Co. (Tianjin, China). CPZ was purchased from a local pharmaceutical factory. TRIS was purchased from Sigma Co. (St. Louis, MO, U.S.A.) All other chemicals used were of analytical grade. The main reagents were dissolved in deionized water.

Animals

Thirty healthy male Sprague-Dawley rats (240-260g) were purchased from the Shanghai Experimental Animal Center (Shanghai, China). The animals were housed in stainless steel cages and provided food and water *ad libitum*. After one week's acclimatization, the rats were divided randomly into 5 groups, with 6 rats in each group. The control animals were given the same volume of normal saline. Cadmium-intoxicated groups were injected with $CdCl_2$ solutions (0.2, 0.4, 0.8 mg Cd/kg respectively) ip daily for a week, while the CPZ-group received 0.4 mg Cd/kg in association with CPZ (5 mg/kg, ip). One day after the last injection, blood samples were collected in heparin tubes. All animals were sacrificed and the liver, kidney, testis and epididymis (Epid) were detached and weighed.

Determination of Metals in Tissues

The concentration of metals in different tissues was determined with an Atomic Absorption Spectrophotometer (AAS) (AA-6501F, Shimadzu, Japan), based on the method described by Baranowska and his coworkers (Baranowska, Czernicki, and Aleksandrowicz 1995) after the samples were digested by heating with HNO_3 + $HClO^4$ (10 + 1).

Preparation of Hemoglobin Solution

Four adult rats were anesthetized by ether, and blood samples were collected in heparin tubes. The blood was centrifuged at 4000 rpm for 10 min and the plasma was removed. Blood cells were washed with KCl solution (11.5g/L) three times. All the blood cells were mixed together in a 50ml tube, then 2 volumes of TRIS-HCl buffer solution (0.5mol/L, pH 8.0) was added to the tube. The solution was centrifuged at 10000 rpm for 20 min, and the hemoglobin solution (supernatant) was collected and stored at 4℃.

Determination of Tissue MT

The tissue MT was detected by cadmium-saturation method (Jin, *et al.*, 1993), with following modifications: samples (about 1g) were homogenized in four volumes of 0.5 mol/L TRIS-HCl buffer solution (pH 8.0), then centrifuged at a speed of 10,000 rpm for 90 min at 4℃. An aliquot of 0.2ml supernatant of each sample was put into 10mL tube. Then 2.2mL TRIS-HCl buffer was added to each tube. After mixing the solutions, 1.0mL $CdCl_2$ solution (1μg Cd/mL) was added into each tube, mixed again and incubated at room temperature for 10 min to saturate the MT binding sites with Cd. The excess cadmium was removed by adding 0.2mL prepared hemoglobin solution, then heated in a 80℃ water bath for 5 min and centrifuged at 5000 rpm for 10 min. This procedure was repeated three times. The Cd concentration of the supernatant from the last centrifugation was determined by AAS. The content of MT in each sample was calculated by assuming that 1 mol MT can bind 6 mols of cadmium, and 1m mol MT = 6100mg.

Statistical Analysis

t-Test was used for data analysis in this study at levels of significance $P<0.05$ and $P<0.01$, according to Sigma Stat.

RESULTS

Effects of Cadmium on Organ Coefficients

The organ coefficients of kidney, testis and epididymis in 0.8 mg Cd/kg group changed significantly after 7 days relative to the control group. However, the organ coefficient of the liver in each dosage group was not markedly affected by cadmium intoxication, and CPZ treatment didn't change the organ coefficient in this study (Table 1).

TABLE 1

Effects of Cadmium on Organ Coefficients in Male Rats ($\bar{x} \pm s$)

Treatment	No of animal	Liver/Body Wt. (%)	Kidney/Body Wt.	Testis/Body Wt.	Epid/Body Wt.
Control	6	3.26±0.25	0.65±0.02	1.18±0.16	0.17±0.04
0.2mg Cd/kg	6	3.41±0.16	0.65±0.03	1.18±0.12	0.15±0.03
0.4mg Cd/kg	6	3.35±0.24	0.64±0.02	1.22±0.04	0.18±0.03
0.8mg Cd/kg	6	3.50±0.44	0.78±0.03[a]	0.93±0.05[b]	0.12±0.01[b]
CPZ+0.4mg Cd/kg	6	3.34±0.12	0.67±0.02	1.26±0.06	0.16±0.02

[a] $P<0.0001$; [b] $P<0.05$, compared with control.

Effects of Cadmium on Tissue MT

Table 2 shows the MT contents in both liver and kidney after different treatments. Cadmium intoxication with the lowest dosage of 0.2mg Cd/kg induced a significant increase of MT in both liver and kidney. There was a dose-response relationship in MT induced by Cd. MT in liver was much higher than that in kidney after 7 days injection of cadmium. CPZ intervention seemed to decrease the liver MT, but this was not statistically significant. However, CPZ treatment significantly increased

the MT content in kidney.

TABLE 2

Effects of Cadmium on Tissue MT in Male Rats ($\bar{x} \pm s$) (μg/g Tissue)

Treatment	No of Animal	Liver MT	Kidney MT
Control	6	23.1 ± 6.4	25.5 ± 11.7
0.2mg Cd/kg	6	344.5 ± 106.2 [a]	71.6 ± 4.6 [a]
0.4mg Cd/kg	6	623.9 ± 153.0 [a]	77.8 ± 7.9 [a]
0.8mg Cd/kg	6	961.8 ± 66.0 [a]	583.0 ± 110.0 [a]
CPZ + 0.4mg Cd/kg	6	563.1 ± 152.8 [a]	106.8 ± 22.0 [a,b]

[a] $P < 0.001$, compared with control. [b] $P < 0.05$, compared with 0.4mg Cd/kg group.

Effects of Cadmium on Metals in Liver

Contents of Cd, Zn, Cu and Ca in liver after cadmium intoxication are illustrated in Table 3. There was a remarkable increase in the cadmium content in liver with an increase of cadmium dosage. Cadmium treatment significantly increased the content of Zn in liver with a dose-response relationship. There was some increase in liver Cu and Ca content with the increase in cadmium dosage, although there was no statistical significance. CPZ treatment remarkably decreased the cadmium content in liver from 49.41 ± 3.47 to 44.72 ± 5.44 μg/g tissue, and increased Zn in liver from 47.15 ± 3.33 to 50.14 ± 2.96 μg/g tissue. There was also an increase of Cu in liver after CPZ intervention, but there was no significant difference between CPZ-treated and Cd-intoxicated group (0.4mg Cd/kg).

TABLE 3

Contents of Liver Metals after Cd Intoxication in Male Rats ($\bar{x} \pm s$) (μg/g Tissue)

Treatment	Number of Rats	Cd	Zn	Cu	Ca
Control	6	0.08 ± 0.01	21.74 ± 0.69	4.22 ± 0.27	2.63 ± 1.10
0.2mg Cd/kg	6	19.13 ± 2.29 [a]	30.34 ± 4.66 [a]	4.81 ± 0.28 [a]	3.71 ± 1.19
0.4mg Cd/kg	6	49.41 ± 3.47 [a]	47.15 ± 3.33 [a]	5.16 ± 0.50 [a]	3.40 ± 0.43
0.8mg Cd/kg	6	88.19 ± 5.32 [a]	66.23 ± 12.35 [a]	4.36 ± 1.40 [a]	3.51 ± 0.62
CPZ + 0.4mg Cd/kg	6	44.72 ± 5.44 [a,b]	50.14 ± 2.96 [a,b]	5.67 ± 0.49 [a]	3.01 ± 0.88

[a] $P < 0.01$, compared with control. [b] $P < 0.05$, compared with 0.4mg Cd/kg group.

Effects of Cadmium on Metals in Kidney

The contents of Cd, Zn, Cu and Ca in kidney after cadmium intoxication were illustrated in Table 4. There was a remarkable increase of cadmium content in kidney with increasing levels of cadmium intoxication. Cadmium treatment significantly increased the contents of Zn and Cu in kidney. There was a significant decrease of kidney Ca with an increase in cadmium dosage. However, CPZ treatment increased the kidney Ca and Cu in this study.

TABLE 4

Contents of Kidney Metals after Cd Intoxication in Male Rats ($\bar{x} \pm s$) (μg/g Tissue)

Treatment	Number of Rats	Cd	Zn	Cu	Ca
Control	6	0.05 ± 0.02	22.94 ± 0.69	7.09 ± 0.97	12.18 ± 4.03
0.2mg Cd/kg	6	6.02 ± 0.83 [a]	25.94 ± 2.72 [a]	9.08 ± 0.50 [a]	11.46 ± 3.38
0.4mg Cd/kg	6	11.50 ± 1.92 [a]	27.47 ± 2.81 [a]	9.57 ± 0.93 [a]	8.35 ± 1.95 [a]
0.8mg Cd/kg	6	47.36 ± 17.78 [a]	27.73 ± 2.79 [a]	10.40 ± 0.30 [a]	6.76 ± 1.48 [a]
CPZ + 0.4mg Cd/kg	6	12.87 ± 1.85 [a]	27.97 ± 1.94 [a]	10.89 ± 0.89 [a,b]	14.41 ± 5.36 [a,b]

[a] $P < 0.01$, compared with control. [b] $P < 0.05$, compared with 0.4mg Cd/kg group.

Effects of Cadmium on Metals in Testis

Contents of Cd, Zn, Cu and Ca in testis after cadmium intoxication were illustrated in Table 5. There was a remarkable increase of cadmium content in testis with increasing cadmium intoxication. Cadmium treatment didn't significantly change the content of Zn and Cu in testis. However, there was a remarkable increase of testis Ca with increasing cadmium dosage.

TABLE 5

Contents of Testis Metals after Cd Intoxication in Male Rats ($\bar{x} \pm s$) (μg/g Tissue)

Treatment	Number of Rats	Cd	Zn	Cu	Ca
Control	6	0.18 ± 0.03	37.05 ± 1.86	1.15 ± 0.23	95.28 ± 45.06
0.2mg Cd/kg	6	0.523 ± 0.06 [a]	31.78 ± 5.19	0.97 ± 0.14	164.38 ± 62.26
0.4mg Cd/kg	6	0.55 ± 0.08 [a]	31.65 ± 9.27	1.02 ± 0.13	215.82 ± 95.95 [a]
0.8mg Cd/kg	6	1.88 ± 0.52 [a]	27.60 ± 3.65 [a]	0.98 ± 0.06	1137.78 ± 458.03 [a]

[a] $P < 0.01$, compared with control.

Effects of Cadmium on Metals in Blood

Content of Cd, Zn, Cu and Ca in blood after cadmium intoxication are listed in Table 6. There was a remarkable increase in cadmium content in blood with increasing cadmium intoxication. Cadmium treatment didn't markedly change the concentrations of Zn, Cu and Ca in blood in this study.

In the present study, the authors found that cadmium accumulated mainly in the liver and kidney after a week of cadmium exposure, followed by the testis. Blood cadmium was much lower than that of these organs. This phenomenon may be related to the biological synthesis and distribution of MT, because liver is an important organ in which MT synthesis is induced (Chan *et al.*, 1992). Shaikh and his coworkers suggested that to some extent, MT in the extracellular fluids, might be regarded as an index of cadmium toxicity (Shaikh and Hirayama, 1979). It was reported that pretreatment with a low concentration of cadmium induced the MT gene expression in cultured rat liver cells, which provided a protective effect against cadmium-induced DNA damage (Coogan *et al.*, 1994). MT, a metal-binding protein is associated with tolerance to cadmium. However, Cd when combined with MT is difficult to be excluded from tissues. Therefore, the homeostasis of essential metals such as Ca, Zn,

and Cu may be affected by Cd because of the similar ionic radius between them (Skocynska, Smolik, and Milian 1994; Kojima *et al.*, 1992). The data of this article showed that there were significant changes of these tissue metals after Cd-intoxication, which supports the hypothesis that loss of metal balance in tissues may contribute to Cd toxicity in different organs.

TABLE 6

Concentrations of Blood Metals after Cd Intoxication in Male Rats ($\bar{x} \pm s$) (μg/mL)

Treatment	Number of Rats	Cd	Zn	Cu	Ca
Control	6	0.04±0.01	6.69±0.58	1.91±0.30	40.38±2.24
0.2mg Cd/kg	6	0.14±0.13[a]	7.75±0.79	1.69±0.32	36.69±7.16
0.4mg Cd/kg	6	0.21±0.03[a]	6.94±1.52	1.65±0.51	41.46±8.73
0.8mg Cd/kg	6	0.31±0.08[a]	6.41±0.44	1.43±0.36[b]	42.70±4.77
CPZ+0.4mg Cd/kg	6	0.23±0.06[a]	7.25±0.42	1.59±0.26	43.84±7.15

[a] $P<0.01$, [b] $P<0.05$, compared with control.

It was reported that calmodulin antagonists protected rodents from Cd toxicity by inhibiting the abnormal activation of CaM-dependent systems. In a previous study, we found that CPZ increased the excretion of Cd from urine and reduced Cd-induced kidney damage (Yang and Yang, 1997). The mechanism of protection by CPZ may be related to: (i) CPZ inhibition of abnormal activation of CaM-dependent enzymes, (ii) CPZ stimulation of MT gene express in tissues, causing an increase in MT that could combine with cadmium, then increased the tolerance to cadmium toxicity. In the present study, these data showed that CPZ treatment seemed to decrease the content of MT in liver and increase the content of MT in kidney, which may be an important process to increase the excretion of Cd from urine. CPZ treatment also reversed the changes of essential metals in different tissues in this study. The reversal of tissue metals might be responsible for the protective effect of CPZ on Cd-induced toxicity. We also found that the cadmium content in liver decreased significantly after CPZ-treatment. The decrease of cadmium load in tissues also played an important role in the protective mechanism of CaM antagonists. In conclusion, the mechanism of cadmium poisoning is a complex process and further studies are needed.

ACKNOWLEDGEMENT

The authors are grateful to Professor Chih-Ying Feng for his great contribution to amending this manuscript.

REFERENCES

Baranowska, I., Czernicki, K., and Aleksandrowicz R. (1995). The analysis of lead, cadmium, zinc, copper and nickel content in human bones from the upper Silesian industrial district. *Sci Total Environ*. **159** (2-3), 155-162.

Chan, H. M., Satoh, M, Zalups, R. K., and Cherian, M. G. (1992). Exogenous metallothionein and renal toxicity of cadmium and mercury in rats. *Toxicology* **76**(1), 15-26.

Coogan, T. P., Bare, R. M., Bjornson, E. J., and Waalkes, M. P. (1994). Enhanced metallothionein gene expression is associated with protection from cadmium-induced genotoxicity in cultured rat liver cells. *J. Toxicol. Environ. Health* **41** (2), 233-245.

Jin, N. Z., Kimura, M., Yokoi, K., and Itokawa, Y. (1993). A gel filtration high-performance liquid chromatographic method for determination of hepatic and renal metallothionein of rat and in comparison

with the cadmium-saturation method. *Biological Trace Element Research* **36**(2), 183-190.
Kojima, S., Sugimura, Y., Hirukawa, H., Hirukana, H., Kiyozumi, M., Shimada, H., and Funakoshi, T. (1992). Effects of dithiocarbamates on testicular toxicity in rats caused by acute exposure to cadmium. *Toxicol. Appl. Pharmacol.* **116**(1), 24-29.
Robert, J., Niewenhuis, J. N., and Prozialeck, W. C. (1987). Calmodulin inhibitors protect agaist cadmium induced testicular damage in mice. *Biol. Reproduc.* **37**, 127.
Shiraishi, N., Rehm, S., and Waalkes, M. P. (1994). Effect of chlorpromazine pretreatment on cadmium toxicity in the male Wistar (WF/NCr) rat. *J. Toxicol. Environ. Health* **42**(2), 193-208.
Shiraishi, N. and Rehm, S. (1994). Enhancement of metallothionein gene expression in male Wistar (WF/NCr) rats by treatment with calmodulin inhibitors: potential role of calcium regulatory pathways in metallothionein induction. *Toxicol. Appl. Pharmacol.* **125**(1), 97-103.
Shaikh, Z. A. and Hirayama, K. (1979). Metallothionein in the extracellular fluids as an index of cadmium toxicity. *Environ. Health Perspect.* **28**, 267-271.
Skocynska, A., Smolik, R., and Milian, A. (1994). The effect of combined exposure to lead and cadmium on the concentration of zinc and copper in rat tissues. *Int. J. Occup. Med. Environ. Health* **7**(1), 41-49.
Sutoo, D., Akiyama, K., and Imamiya, S. (1990). A mechanism of cadmium poisoning: the cross effect of calcium and cadmium in the calmodulin-dependent system. *Arch. Toxicol.* **64**(2), 161-164.
Yang, X. F. and Yang, Y. N. (1997). Protective effects of calcium antagonists on cadmium-induced toxicity in rats. *Biomed. & Environ. Sci.* **10**, 402-407.

(*Received June* 14, 1999 *Accepted November* 28, 1999)