Effects of Three Dithiocarbamates on Tissue Distribution and Excretion of Cadmium in Mice

Shoji KOJIMA,* Minoru KAWAGOE, Morio KIYOZUMI, and Hideaki SHIMADA

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1 Oe-honmachi, Kumamoto, 862, Japan.
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N-Benzyl-D-glucamine dithiocarbamate (BGD), N-p-hydroxymethylbenzyl-D-glucamine dithiocarbamate (HBGD), and N-p-carboxybenzyl-D-glucamine dithiocarbamate (CBGD) were compared for their relative efficacies in the distribution and excretion of cadmium in mice exposed to cadmium. Mice were injected intraperitoneally with $^{109}CdCl_2$ (1 mg of Cd/kg and 2 μCi of ^{109}Cd/one animal). Three days later, they were injected with chelating agents (400 μmol/kg) every other day for 2 weeks. After injections of BGD and HBGD, cadmium was excreted mainly in the feces through the bile, and the fecal excretion of cadmium by HBGD was significantly higher than that by BGD or CBGD. These chelating agents increased the urinary excretion of cadmium to a small extent. The hepatic cadmium content was decreased only after HBGD injection. Also, the injection of HBGD caused a much greater decrease in renal cadmium content than did BGD or CBGD. These chelating agents did not result in the redistribution of cadmium to the brain, testes, or heart. The growth of mice was only slightly retarded by injections of these chelating agents. The results of this study indicate that the injection of HBGD to mice pretreated with cadmium can remove cadmium from the body, mainly through fecal excretion, without redistribution of cadmium to other tissues such as the brain, testes, and heart, more effectively than BGD or CBGD.

Keywords cadmium; tissue distribution; excretion; dithiocarbamate; chelate effect; partition coefficient

A single injection of cadmium into animals may result in a number of lesions in various organs, such as the liver, testes, and sensory ganglia.[1] It is also known that the kidney is the most sensitive organ to long-term exposure to cadmium, *i.e.*, the critical organ for this type of exposure.[2,3] Chelation therapy for cadmium has been effective in preventing cadmium-induced diseases. Various dithiocarbamate compounds have been used as antidotes for cadmium intoxication in animals. The injections of dimethyldithiocarbamate (DMD),[4] diethyldithiocarbamate (DED),[4-7] and diisopropyldithiocarbamate (DPD)[4] in mice pretreated with $CdCl_2$ greatly decreased hepatic and renal cadmium burdens, but led to marked redistribution of a portion of the cadmium to the brain, testes, and heart. Repeated intraperitoneal injection of dihydroxyethyldithiocarbamate[6] and *N*-methyl-D-glucamine dithiocarbamate (MGD),[5,8] in mice after cadmium exposure resulted in a substantial reduction in cadmium levels in both the liver and kidney without redistribution of cadmium to the brain, testes, and heart. *N*-Cyclohexyl-*N*-sulfonatoalkyl dithiocarbamate[9] significantly decreased cadmium levels in the liver and kidney of mice pretreated with cadmium. Recently, our studies indicated that the new dithiocarbamate derivatives, *N*-benzyl-D-glucamine dithiocarbamate (BGD),[10-12] *N*-*p*-methylbenzyl-D-glucamine dithiocarbamate (MBGD),[13] and *N*-*p*-isopropylbenzyl-D-glucamine dithiocarbamate (PBGD),[13] were more effective than other dithiocarbamates in decreasing cadmium concentrations in the liver and kidney in cadmium-pretreated rats without redistribution of cadmium to tissues, such as the brain, testes, and heart.

In order to develop better chelating agents to mobilize cadmium from the body, we studied the comparative effects of BGD, *N*-*p*-hydroxymethylbenzyl-D-glucamine dithiocarbamate (HBGD), and *N*-*p*-carboxybenzyl-D-glucamine dithiocarbamate (CBGD), which were newly synthesized by us, on the excretion and tissue distribution of cadmium in mice exposed to cadmium.

Experimental

Materials ^{109}Cd (specific activity, 1574 mCi/mg) was obtained from New England Nuclear (Boston, Mass). Cadmium chloride was obtained from Wako Pure Chemical Ind. (Osaka). The sodium salt of BGD was synthesized by the method reported in our previous paper.[10] The sodium salts of HBGD and CBGD were synthesized as described elsewhere.[14] Structural formulas of dithiocarbamates used are shown in Fig. 1. All other chemicals were of a reagent grade.

Distribution and Excretion Studies Male ddY mice, weighing 20—25 g, were injected intraperitoneally with $^{109}CdCl_2$ (1 mg of Cd/Kg and 2 μCi of ^{109}Cd/one animal) in 0.3 ml saline and housed in individual metabolic cages with drinking water and diet (Nosan Lab Chow) *ad libitum*. Three days later, the mice received 7 intraperitoneal injections of saline or a chelating agent (400 μmol/kg) every other day for 2 weeks. Each chelating agent was dissolved in 0.2 ml saline and these solutions were prepared fresh every day. The body weights of the mice were measured every other day. Urine and fecal samples were collected from each mouse daily and were counted in an Aloka auto-well gamma counter (model ARC 300) for ^{109}Cd radioactivity. The mice were killed with urethane 48 h after the final administration and various tissues were removed for ^{109}Cd counting.

***In Situ* Mouse Biliary Excretion Experiment** Male ddY mice, weighing 30—35 g, were injected intraperitoneally with $^{109}CdCl_2$ (1 mg of Cd/kg and 2 μCi of ^{109}Cd/one animal) in 0.3 ml of saline and housed in individual metabolic cages with drinking water and diet *ad libitum*. After 24 h, the mice were anesthetized with urethane (1 g/kg, intraperitoneally). The bile duct was exposed through a midline abdominal incision and cannulated with a 5-cm length of polyethylene tubing (PE 5). The mice were injected intraperitoneally with saline or a chelating agent (400 μmol/kg) in 0.2 ml of saline. Bile and urine samples were collected for an experimental period of 3 h. The levels of ^{109}Cd radioactivity in the bile and urine were determined using the gamma counter.

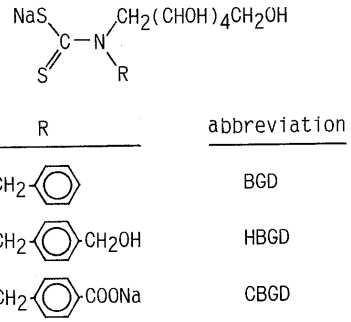

Fig. 1. Structures of Dithiocarbamates Used

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Determination of Partition Coefficients The *n*-octanol/aqueous partition coefficient of each chelating agent–cadmium complex was determined according to the method of our previous paper[10] using 0.1 M Tris buffer (pH 7.4) as the aqueous solvent. The partition coefficients were expressed as $\log_{10}$ (cpm of ^{109}Cd in the *n*-octanol phase/cpm of ^{109}Cd in the aqueous phase).

Determination of LD_{50} LD_{50} values of HBGD and CBGD in male ddY mice were determined according to the method of Behrens and Kärber.[15]

Statistical Analysis Data were compared by analysis of variance. When the analysis indicated a significant difference, the treated groups were compared to controls by using Duncan's new multiple test.

Results and Discussion

The effects of chelating agents, such as BGD, HBGD, and CBGD, on the fecal and urinary excretion of cadmium in mice pretreated with cadmium were investigated (Fig. 2). The control mice excreted 3.14 and 0.05% of the dose in feces and urine, respectively, during the two-week period. There were large increases in the fecal excretion of cadmium in mice treated with BGD and HBGD during the two weeks. The total fecal excretion of cadmium after BGD of HBGD treatment for the two weeks was about 3.7 and 11.2 times higher, respectively, than that of the control. However, CBGD treatment did not significantly increase the fecal excretion of cadmium. The increased fecal excretion of cadmium by HBGD was significantly larger than that by BGD or CBGD. Gale *et al.*[16] reported that when given a total of seven injections of MGD at a much higher dose (4.4 mmol/kg) to mice, about 30% of the administered cadmium was excreted in the feces. A regimen of seven injections of HBGD at a much lower dose (400 μmol/kg) promoted the fecal excretion of almost 35% of the administered cadmium. Such an enhancing effect of HBGD on the fecal excretion of cadmium is considered to be larger than that of MGD. There was a gradual increase in the urinary excretion of cadmium in mice treated with BGD, HBGD, or CBGD during the two weeks, indicating total urinary cadmium excretion of less than about 4% of the dose.

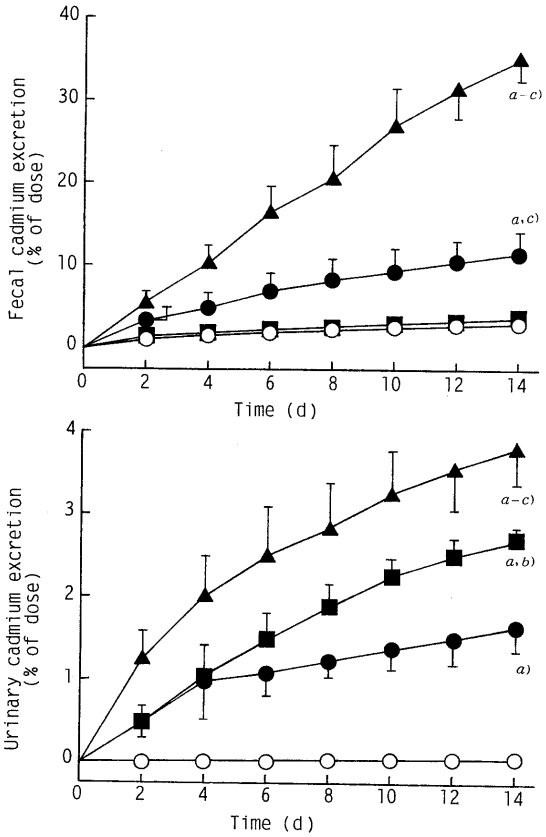

Fig. 2. Cumulative Fecal and Urinary Excretion of Cadmium during Treatment with Chelating Agents for Two Weeks in Mice Pretreated with Cadmium

The mice were injected intraperitoneally with $^{109}CdCl_2$ (1 mg Cd/kg and 2 μCi ^{109}Cd/one animal). Three days later they were injected intraperitoneally with saline or chelating agents (400 μmol/kg) every other day for two weeks. Fecal and urine samples were collected in metabolic cages and counted for ^{109}Cd radioactivity. The values represent the mean ± S.D. for 3 to 5 animals. ○, control; ●, BGD; ▲, HBGD; ■, CBGD. *a*) significantly different from control, $p<0.05$. *b*) Significantly different from BGD, $p<0.05$. *c*) Significantly different from CBGD, $p<0.05$.

The effects of BGD, HBGD, and CBGD on biliary and urinary excretion of cadmium in mice pretreated with cadmium, 24 h earlier, are shown in Table I. The results showed that the major route of excretion of cadmium after injection of the chelating agents other CBGD to cadmium-treated mice was *via* the bile. Biliary excretion of cadmium was significantly increased by the use of BGD or HBGD. However, biliary excretion of cadmium after injection of HBGD was significantly larger than that of BGD. In addition, HBGD and CBGD increased the urinary excretion of cadmium to a small extent. As shown in Fig. 2, the injections of BGD and HBGD mobilized cadmium from the mouse body mainly through fecal excretion. These results confirm the data on increased biliary excretion of cadmium by the injections of BGD and HBGD after

TABLE I. Effects of Chelating Agents on Biliary and Urinary Excretion of Cadmium in Mice Pretreated with Cadmium 24 h Earlier

Chelating agent	Cadmium excreted/3 h (% of dose)[a]	
	Bile	Urine
Control	0.02 ± 0.01	0.02 ± 0.01
BGD	4.07 ± 0.56[b]	0.13 ± 0.08
HBGD	9.43 ± 3.27[b,c]	1.12 ± 0.50[b,c]
CBGD	0.16 ± 0.08[c]	0.62 ± 0.12[b,c]

The mice were injected intraperitoneally with $^{109}CdCl_2$ (1 mg Cd/kg and 2 μCi ^{109}Cd/one animal). After 24 h, they were injected intraperitoneally with saline or chelating agents (400 μmol/kg) and bile and urine samples were collected for 3 h. *a*) The values represent the mean ± S.D. for 3 to 6 animals. *b*) Significantly different from control, $p<0.05$. *c*) Significantly different from BGD, $p<0.05$.

TABLE II. Effects of Chelating Agents on Tissue Distribution of Cadmium in Mice

Tissue	Cadmium (% of dose)[a]			
	Control	BGD	HBGD	CBGD
Liver	46.46 ± 5.49	43.64 ± 4.02	25.96 ± 6.86[b]	42.21 ± 1.25
Kidney	7.02 ± 0.62	4.02 ± 0.31[b]	1.77 ± 0.25[b,c]	2.78 ± 0.24[b–d]
Pancreas	2.40 ± 0.24	2.32 ± 0.32	2.39 ± 0.19	2.53 ± 0.39
Heart	0.24 ± 0.02	0.26 ± 0.03	0.25 ± 0.04	0.25 ± 0.03
Testes	0.13 ± 0.04	0.23 ± 0.11	0.13 ± 0.03	0.35 ± 0.11
Lung	0.30 ± 0.04	0.28 ± 0.04	0.28 ± 0.06	0.36 ± 0.02
Spleen	0.58 ± 0.10	0.54 ± 0.16	0.59 ± 0.24	0.40 ± 0.04
Brain	0.11 ± 0.04	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.00

The mice were injected intraperitoneally with $^{109}CdCl_2$ (1 mg Cd/kg and 2 μCi ^{109}Cd/one animal). Three days later they were injected intraperitoneally with saline or chelating agents (400 μmol/kg) every other day for two weeks. The mice were killed 48 h after the last injection and the tissue distribution of cadmium was determined from radioactivity. *a*) The values represent the mean ± S.D. for 3 to 5 animals. *b*) Significantly different from control, $p<0.05$. *c*) Significantly different from BGD, $p<0.05$. *d*) Significantly different from HBGD, $p<0.05$.

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cadmium treatment, and they also suggest that the cadmium excreted mainly through the bile after injections of these chelating agents is reabsorbed only in very small amounts from the intestinal tracts.

The results of the tissue distribution of cadmium in mice at the end of the experiment are shown in Table II. Only HBGD among the chelating agents used here significantly reduced the cadmium deposited in the liver. The reducing effect of HBGD on the hepatic cadmium levels was almost the same as that of MBGD or PBGD, which was reported in our previous paper.[17] The percentage of cadmium in the kidney was significantly reduced after injections of BGD, HBGD, and CBGD when compared with the control. The reducing effect of HBGD on the renal cadmium content was much larger than that of BGD or CBGD. We reported that MBGD and PBGD did not significantly reduce the renal cadmium content in mice pretreated with cadmium.[17] Of BGD, HBGD, CBGD, MBGD, and PBGD, all of which were synthesized by us, HBGD was the most effective in reducing the renal and hepatic cadmium levels in mice after cadmium treatment.

Table III shows the partition coefficients of the complexes of cadmium with the dithiocarbamates used here together with some of the values reported in our previous paper.[13] In contrast to MBGD and PBGD, which yielded lipid-soluble complexes with cadmium, the complexes of cadmium with HBGD and CBGD had greater lipophobicity, and the complex of cadmium with BGD indicated intermediate lipophobicity. The dithiocarbamate analogs which form cadmium complexes with partition coefficients ranging from about -5 to 0, particularly HBGD and CBGD, showed much larger reducing effects on renal cadmium content. In addition, the dithiocarbamates which form cadmium complexes with partition coefficients ranging from about -2 to 3, particularly HBGD, MBGD, and PBGD, resulted in a remarkable reduction of hepatic cadmium content. Jones and Jones[18] have suggested that changes in the relative hydrophobic/hydrophilic character of the groups attached to the nitrogen atom of dithiocarbamates may play an important role in determining the antidotal activity of the compounds. The results of the present study also suggest that the pattern of tissue distribution and excretion of cadmium after treatment with chelating agents is related to the partition coefficients of the cadmium–dithiocarbamate complexes.

The chelating agents used here did not promote the redistribution of cadmium to brain, testes, and heart. Gale *et al.*[4-6] reported that injection of dithiocarbamates, such as DED, DMD, and DPD, to mice pretreated with cadmium extensively reduced hepatic and renal cadmium burdens, and also caused redistribution of cadmium to the brain, testes, and heart. The partition coefficients of the cadmium complexes with BGD, HBGD, and CBGD were $\log_{10}P$ values of 0.30, -2.49, and <-5.15, respectively (Table III). Thus, an inability of the complexes of cadmium with BGD, HBGD, and CBGD to penetrate the blood-brain barrier was suggested by their low partition coefficients.

Figure 3 shows the relationship between the hepatic cadmium content and fecal cadmium excretion, or the renal cadmium content and urinary cadmium excretion. The results suggest that hepatic cadmium is removed largely by the fecal route, while renal cadmium is removed largely by the urinary route. The major route of excretion of cadmium following injections of these chelating agents in cadmium-treated mice was the feces. The urinary excretion of cadmium following treatment with the chelating

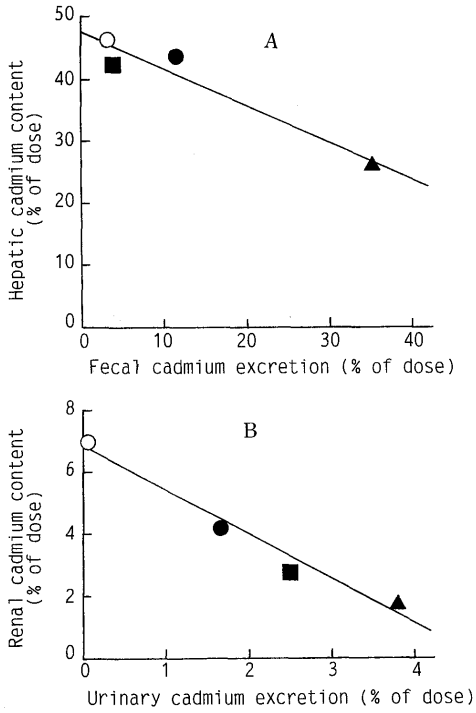

Fig. 3. Relationship between Hepatic Cadmium Content and Fecal Cadmium Excretion or Renal Cadmium Content and Urinary Cadmium Excretion in Mice Injected with Chelating Agents after Cadmium Pretreatment

○, control; ●, BGD; ▲, HBGD; ■, CBGD. A: $r=-0.962$, $p<0.02$. B: $r=-0.986$, $p<0.02$.

TABLE III. Partition Coefficient of the Cadmium Ion and Cadmium Complexes with Chelating Agents in *n*-Octanol/Aqueous System

Reaction mixture	Partition coefficient[a] 0.1 M Tris buffer
Cd ion only[b]	-3.13 ± 0.11
Cd+PBGD[b]	3.16 ± 0.21
Cd+MBGD[b]	1.35 ± 0.02
Cd+BGD[b]	0.30 ± 0.01
Cd+HBGD	-2.49 ± 0.01
Cd+CBGD	<-5.15

a) Expressed as $\log_{10}$ (cpm in *n*-octanol phase/cpm in aqueous phase) at 37 °C. Each value represents the mean ± S.D. for 3 independent experiments. *b*) The values were cited from reference 13.

TABLE IV. LD_{50} Values of Chelating Agents in Mice

Chelating agent	LD_{50}	
	mmol/kg	mg/kg
BGD[a]	11.16	4320
HBGD	26.50	10570
CBGD	>30.0	>12390
MBGD[a]	4.21	1690
PBGD[a]	2.61	1119

Chelating agents were administered intraperitoneally to groups of 6 mice per dose. Survival was recorded at the end of 14 d. *a*) The values were from reference 17.

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agents is considered to be a fraction of the total loss of cadmium from the kidneys.

The body weight gain of mice was not significantly affected by the administration of BGD, HBGD, and CBGD. The LD_{50} values for BGD, HBGD, and CBGD are shown in Table IV. The LD_{50} values of HBGD and CBGD were lower than those of BGD, MBGD, and PBGD.

In conclusion, the present study reveals that HBGD is more effective than BGD and CBGD in removing cadmium from the body mainly through fecal excretion. The injection of HBGD to mice treated with cadmium is more effective than BGD or CBGD in decreasing the cadmium content in the liver and kidney without redistribution of cadmium to other tissues, such as the brain, testes, and heart. Therefore the therapeutic effect of HBGD on cadmium-induced renal damage is suggested to be greater than that of BGD or CBGD.

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