Effects of Several Dithiocarbamates on Tissue Distribution and Excretion of Inorganic Mercury in Rats

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The effects of various chelating agents, sodium *N*-benzyl-D-glucamine dithiocarbamate (BGD), sodium *N*-*p*-methylbenzyl-D-glucamine dithiocarbamate (MBGD), sodium *N*-*p*-hydroxymethylbenzyl-D-glucamine dithiocarbamate (HBGD), and *N*-*p*-carboxybenzyl-D-glucamine dithiocarbamate (CBGD), which were newly synthesized, and sodium *N*-methyl-D-glucamine dithiocarbamate (MGD), on the distribution and excretion of inorganic mercury were compared in rats exposed to $HgCl_2$. Rats were injected i.p. with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg) and 30 min or 24 h later, they were injected with a dithiocarbamate (1200 μmol/kg). At 30 min after mercury administration, BGD and MBGD significantly enhanced the biliary excretion of mercury, while CBGD, MGD, and HBGD enhanced the urinary excretion of mercury to a small extent. At 24 h after mercury injection, BGD was the most effective on the biliary excretion of the metal, while MGD and HBGD significantly enhanced the urinary excretion of the metal. All of these dithiocarbamates were effective in mobilizing mercury from the kidney at 30 min after mercury treatment. At 24 h after mercury treatment, HBGD and BGD effectively depressed mercury content in the kidney. These results show that the injection of BGD and HBGD at both 30 min and 24 h after mercury treatment can much more effectively mobilize mercury from the kidney without redistribution of mercury to other tissues, such as brain, heart, and lung, when compared with injection of other chelating agents. The pattern of mobilization and excretion of mercury following treatment with each chelating agent was related to the organic/aqueous partition coefficient of each dithiocarbamate–mercury complex.

Keywords inorganic mercury; tissue distribution; excretion; dithiocarbamate; chelate effect; partition coefficient

Inorganic mercury is predominantly accumulated in the kidney, and affects the morphology and function of the proximal tubules.[1–3] Therefore, it is important to develop an effective chelator therapy for removal of mercury to prevent mercury-induced diseases. The most potent mercury-detoxifying agents are SH-containing compounds, of which D-penicillamine (D-PEN) and 2,3-dimercaptopropanol (BAL) are used in clinical medicine. The practical usefulness of BAL, however, is limited by a low therapeutic index.[4] We reported that single and repeated injections of the new dithiocarbamate derivative, sodium *N*-benzyl-D-glucamine dithiocarbamate (BGD), into rats treated with mercury were effective in decreasing the mercury content in the kidney without redistribution of mercury to tissues such as the brain, heart, and lung and that the reducing effect of BGD on the kidney mercury content was almost the same as that of BAL and much larger than that of D-PEN.[5,6] Recently, we reported that the injection of sodium *N*-*p*-methylbenzyl-D-glucamine dithiocarbamate (MBGD), which was newly synthesized by us, into rats pretreated with cadmium was more effective in decreasing the cadmium concentration in liver than injection of BGD.[7]

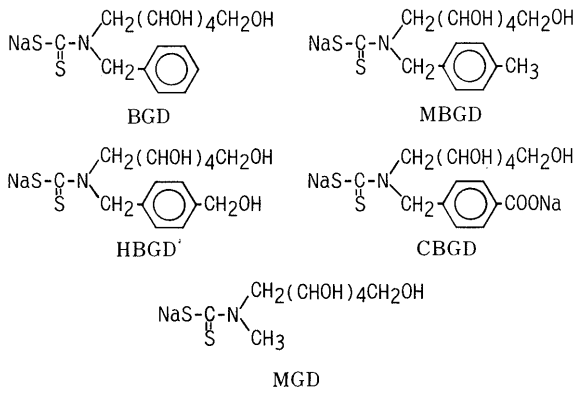

Fig. 1. Structures of Dithiocarbamates Used

In order to develop better chelating agents to mobilize mercury from the body, we studied the comparative effects of BGD, MBGD, sodium *N*-*p*-hydroxymethylbenzyl-D-glucamine dithiocarbamate (HBGD), and sodium *N*-*p*-carboxybenzyl-D-glucamine dithiocarbamate (CBGD), which were newly synthesized by us, as well as sodium *N*-methyl-D-glucamine dithiocarbamate (MGD), on the biliary and urinary excretion and tissue distribution of mercury in rats at 30 min and 24 h after pretreatment with mercuric chloride ($HgCl_2$).

Experimental

Materials $^{203}HgCl_2$ (specific acitivity, 4.59 mCi/mg) was obtained from New England Nuclear (Boston, Mass). $HgCl_2$ was obtained from Wako Pure Chemical Ind. (Osaka). MGD was prepared according to the procedure of Shinobu *et al.*[8] BGD and MBGD were synthesized according to the procedure reported in a previous paper.[9] HBGD and CBGD were synthesized by the use of *N*-*p*-hydroxymethylbenzyl-D-glucamine and *N*-*p*-carboxybenzyl-D-glucamine respectively, according to our method.[9] The products were analyzed, and the results were as follows. HBGD: *Anal.* Calcd for $C_{15}H_{22}NNaO_6S_2$: C, 45.12; H, 5.51; N, 3.51. Found: C, 45.28; H, 5.77; N, 3.70. CBGD: *Anal.* Calcd for $C_{15}H_{20}NNa_2O_7S_2$: C, 41.37; H, 4.41; N, 3.22. Found: C, 41.03; H, 4.39; N, 3.01. Structural formulas of dithiocarbamates used are shown in Fig. 1. All other chemicals were of reagent grade.

Distribution and Excretion Studies Male Wistar rats, weighing 190—220 g, were injected intraperitoneally with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg) in 1.0 ml of physiological saline and housed in individual metabolic cages with drinking water and diet (Nosan Lab Chow) *ad libitum*. At 24 h later, the rats were anesthetized with urethane (1 g/kg intraperitoneally) and the bile duct was cannulated with polyethylene tubing (PE 10) as described previously.[10] The rats were intraperitoneally given saline (control) or a chelating agent (1200 μmol/kg) in 1.0 ml of saline. Mercury was injected intravenously when the chelating agents were administered 30 min after mercury. Bile and urine samples were collected for an experimental period of 5 h. Then, the rats were killed with urethane and various tissues were collected. The levels of ^{203}Hg radioactivity in the bile, urine and tissues were determined using an Aloka auto-well gamma scintillation counter (model ARC 300).

Determination of Partition Coefficients The *n*-octanol/aqueous partition coefficient of each chelating agent-mercury complex was determined according to the procedure in our previous paper using 0.1 M Tris buffer (pH 7.4) as the aqueous solvent.[9] The partition coefficients werer expressed as $\log_{10}$ (cpm of ^{203}Hg in the *n*-octanol phase/cpm of ^{203}Hg in the aqueous phase).

Statistical Analysis The data from the excretion and distribution study were compared by an analysis of variance. When the analysis indicated that a significant difference existed, the treated groups were compared to the control by using Duncan's new multiple test.

Results and Discussion

Table I shows the biliary and urinary excretions of mercury when each chelating agent was injected at 30 min after $HgCl_2$ pretreatment. The major route of excretion of mercury after BGD or MBGD treatment in $HgCl_2$-exposed rats was *via* the bile. The cumulative biliary excretion of mercury in a 5 h period after treatment with BGD or MBGD was remarkably larger than that with CBGD, MGD, or HBGD. CBGD, MGD, and HBGD significantly increased the urinary excretion of mercury, but did not increase the biliary excretion of the metal. HBGD among the chelating agents used showed the largest stimulatory effect on the urinary excretion of mercury.

The tissue distribution of mercury in rats at the end of the experiment is shown in Table II. The contents of mercury deposited in the kidney were significantly decreased by all of these chelating agents. The benzyl-D-glucamine dithiocarbamate analogues (CBGD, HBGD, BGD, and MBGD) greatly reduced the mercury content in the kidney. Diethyl dithiocarbamate caused undesirable redistribution of mercury to brain.[11,12] However, CBGD, HBGD, BGD, and MBGD, which were synthesized by us, did not cause the redistribution of mercury to brain.

The biliary and urinary excretions of mercury in rats injected with the chelating agents at 24 h after $HgCl_2$ pretreatment are shown in Table I. The biliary excretion of mercury was significantly increased by treatment with BGD or MBGD. BGD showed the largest stimulatory effect on the biliary excretion of mercury. The urinary excretion of mercury was significantly increased by treatment with MGD, HBGD, or BGD. The increased urinary excretion caused by the administration of MGD and HBGD at 24 h after $HgCl_2$ pretreatment was greater than that at 30 min after mercury. However, the delayed treatment with these chelating agents showed less effectiveness on the biliary excretion of mercury than that at 30 min after $HgCl_2$ exposure. The differences in the urinary and biliary excretions between 30 min and 24 h after mercury treatment are probably based on the difference in the renal and blood mercury contents before the administration of chelating agents (Table III).

Table IV shows the tissue distribution of mercury at 5 h after injection of the chelating agents into rats pretreated with $HgCl_2$ 24 h earlier. Only HBGD and BGD among these chelating agents significantly reduced the contents of mercury in the kidney, the major site of accumulation of mercury in the body, without the redistribution of mercury to the brain. These results indicate that the injection of these 2 chelating agents can mobilize metallothionein-bound mercury from the rat body even after the synthesis of metallothionein, leading to excretion of the metal through the urine and bile, as reported in our previous paper.[13] The contents of mercury in other organs did not change after treatment with the chelating agents.

The partition coefficients of the complexes of mercury with the chelating agents are listed in Table V. BGD and MBGD yielded lipid-soluble complexes with Hg^{2+}, and the complexes of CBGD, MGD, and HBGD with the metal were lipophobic. The relationship between the biliary or urinary excretion of mercury and the $\log_{10} P$ value of the mercury–chelating agent complex is shown in Fig. 2A and

TABLE I. Effects of Chelating Agents on Biliary and Urinary Excretions of Mercury in Rats Pretreated with Mercury, 30 min and 24 h Earlier

Chelating agent	Mercury excreted/5 h (% of dose)[a]			
	Mercury pretreatment			
	30 min earlier		24 h earlier	
	Bile	Urine	Bile	Urine
Control	1.01±0.16	0.21±0.10	0.48±0.07	1.57±0.42
CBGD	1.94±0.25	1.79±0.26[c]	0.62±0.08	4.58±0.52
MGD	2.01±0.29	2.49±0.41[c]	0.24±0.06	6.04±1.48[b]
HBGD	4.97±1.58	3.75±0.76[c]	1.06±0.39	8.91±0.37[c]
BGD	18.70±2.03[c]	0.68±0.07	9.84±1.65[c]	3.45±0.80
MBGD	19.19±2.61[c]	0.16±0.07	3.33±0.99[c]	1.68±0.25

The rats were injected with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg). At 30 min or 24 h later they were injected intraperitoneally with saline or chelating agents (1200 μmol/kg) and then bile and urine samples were collected for 5 h. *a*) The values represent the mean±S.D. for 3 to 5 animals. Significantly different from the control values: *b*) $p<0.05$; *c*) $p<0.01$.

TABLE II. Effects of Chelating Agents on Tissue Distribution of Mercury in Rats Pretreated with Mercury, 30 min Earlier

Tissue	Mercury (% of dose)[a]					
	Control	CBGD	MGD	HBGD	BGD	MBGD
Liver	13.57±1.25	9.60±1.06[b]	10.07±0.40	8.69±1.21[c]	13.04±0.66	12.78±0.87
Kidney	33.86±0.75	22.47±2.51[c]	25.07±3.00[c]	18.31±1.05[c]	19.41±3.41[c]	22.03±2.62[c]
Spleen	1.30±0.27	0.76±0.20[b]	0.72±0.06[b]	0.64±0.17[c]	0.97±0.03	1.04±0.08
Testes	0.16±0.02	0.22±0.03	0.20±0.03	0.20±0.04	0.18±0.01	0.19±0.01
Heart	0.18±0.02	0.18±0.03	0.15±0.03	0.18±0.02	0.13±0.01	0.12±0.01
Small intestine	1.89±0.42	1.51±0.14	1.51±0.29	1.61±0.43	1.71±0.63	1.25±0.09
Brain	0.08±0.02	0.06±0.01	0.06±0.01	0.07±0.00	0.05±0.01	0.05±0.00
Pancreas	0.58±0.06	0.51±0.07	0.60±0.19	0.49±0.09	0.36±0.13	0.33±0.03
Lung	0.65±0.05	0.69±0.23	0.60±0.09	0.78±0.14	0.63±0.05	0.52±0.06

The rats were injected intravenously with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg). After 30 min, they were injected intraperitoneally with saline or chelating agents (1200 μmol/kg). At the end of the experiment, the rats were killed and the tissue distribution of mercury was determined from the radioactivity. *a*) Each value represents the mean±S.D. for 3 to 5 animals. Significantly different from the control values: *b*) $p<0.05$; *c*) $p<0.01$.

2B. In the treatment with the chelating agents at 30 min and 24 h after mercury exposure, the dithiocarbamates such as BGD and MBGD, which form complexes with mercury having $\log_{10} P$ values of about 2, remarkably enhanced the biliary excretion of mercury resulting in decreased mercury contents in the kidney. Norseth and nordhagen[11] and Aaseth *et al.*[12] have reported that the lipophilic character of mercury–dithiocarbamate complex seems to cause the redistribution of mercury to the brain. However, the mobilization of mercury from the kidney by BGD or MBGD did not promote the redistribution of mercury to the brain in spite of the higher lipophilicity of the complexes of mercury with BGD and MBGD. At 30 min and 24 h after mercury exposure, the dithiocarbamates such as CBGD, MGD, and HBGD, which form complexes with mercury having $\log_{10} P$ values of about -3 to 0, enhanced the urinary excretion of mercury. Jones and Jones[14] have suggested that changes in the relative hydrophobic/hydrophilic character of the groups attached to the nitrogen atom of dithiocarbamates may be important in determining the antidotal activity of the compounds. Figure 2C shows the relationship between the total excretion of mercury and the $\log_{10} P$ value of the mercury–chelating agent complex. At 30 min after mercury exposure, BGD and MBGD, which form complexes with mercury having $\log_{10} P$ values of about 2, were the most effective on the excretion of mercury. At 24 h after mercury exposure, HBGD and BGD, which form complexes with mercury having $\log_{10} P$ values of about 0 to 2.0, enhanced the excretion of mercury. The results of the present study also suggest that the pattern of distribution and excretion of mercury after treatment with these chelating agents is related to the partition coefficients of the mercury–dithiocarbamate complexes and that BGD, in which the complex with mercury exhibits a $\log_{10} P$ value of about 2.0,

Table III. Tissue Distribution of Mercury in Rats at 30 min and 24 h after Mercury Treatment

Tissue	Mercury (% of dose)[a]	
	Time after mercury	
	30 min	24 h
Liver	11.22±2.87	6.49±1.15
Kidney	24.64±2.38	41.24±1.65
Spleen	3.84±1.38	0.35±0.04
Testes	0.15±0.02	0.23±0.03
Heart	0.17±0.02	0.07±0.01
Small intestine	0.79±0.09	1.47±0.09
Brain	0.06±0.00	0.03±0.01
Pancreas	0.12±0.00	0.66±0.13
Lung	0.51±0.09	0.26±0.04
Blood[b]	24.97±1.41	3.60±0.34

The rats were injected intravenously (30 min) or intraperitoneally (24 h) with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg). After 30 min or 24 h, the rats were killed and the tissue distribution of mercury was determined from the radioactivity. *a*) Each value represents the mean±S.D. for 3 animals. *b*) Blood value was estimated as 9% of body weight.[15]

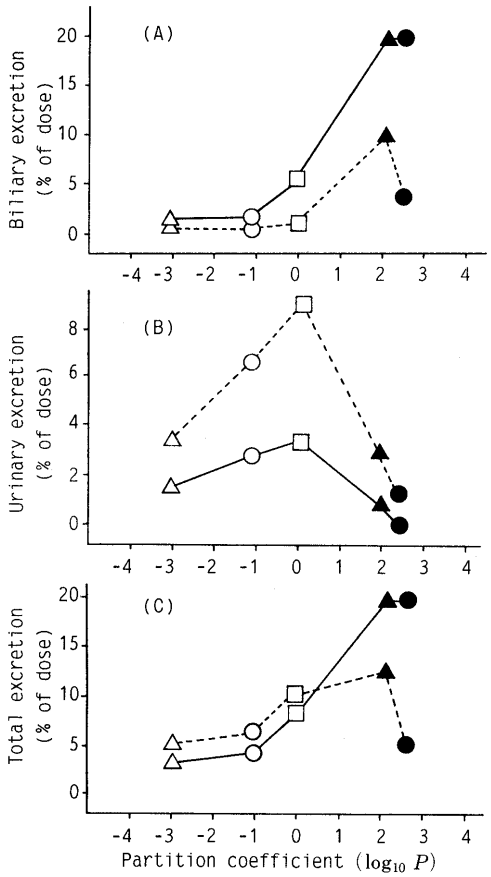

Fig. 2. Relationship between Biliary (A), Urinary (B), or Total (C) Excretion of Mercury and Partition Coefficient of Mercury–Chelating Agent Complex

△, CBGD; ○, MGD; □, HBGD; ▲, BGD; ●, MBGD. ——, 30 min; ------, 24 h.

Table IV. Effects of Chelating Agents on Tissue Distribution of Mercury in Rats Pretreated with Mercury, 24 h Earlier

Tissue	Mercury (% of dose)[a]					
	Control	CBGD	MGD	HBGD	BGD	MBGD
Liver	6.71±0.96	4.34±0.28[b]	5.25±0.53	4.66±0.39	5.87±0.24	5.28±0.32
Kidney	39.44±2.98	36.55±2.88	34.15±1.84	32.43±1.46[b]	26.14±0.51[c]	36.99±1.03
Spleen	0.24±0.04	0.22±0.01	0.36±0.09	0.38±0.04	0.25±0.04	0.26±0.06
Testes	0.17±0.03	0.13±0.01	0.18±0.03	0.17±0.02	0.17±0.04	0.20±0.01
Heart	0.07±0.01	0.05±0.00	0.07±0.01	0.06±0.01	0.06±0.00	0.00±0.01
Small intestine	1.56±0.29	1.37±0.40	1.20±0.17	1.20±0.17	1.27±0.29	1.23±0.04
Brain	0.03±0.01	0.03±0.00	0.03±0.01	0.03±0.00	0.03±0.00	0.04±0.01
Pancreas	0.74±0.23	0.81±0.17	0.91±0.21	1.35±0.39	0.51±0.22	0.85±0.30
Lung	0.25±0.04	0.27±0.13	0.34±0.11	0.18±0.01	0.20±0.04	0.18±0.01

The rats were injected intraperitoneally with $^{203}HgCl_2$ (300 μg of Hg and 74 kBq of ^{203}Hg/kg). After 24 h, they were injected intraperitoneally with saline or chelating agents (1200 μmol/kg). At the end of the experiment, the rats were killed and the tissue distribution of mercury was determined from the radioactivity. *a*) Each value represents the mean±S.D. for 3 to 5 animals. Significantly different from the control values: *b*) $p<0.05$; *c*) $p<0.01$.

TABLE V. Partition Coefficient of Mercury Ion and Mercury Complexes with Chelating Agents in *n*-Octanol/Aqueous System

Reaction mixture	Partition coefficient[a] 0.1 M Tris buffer
Hg ion only	-1.076 ± 0.006
Hg+CBGD	-3.019 ± 0.044
Hg+MGD	-1.245 ± 0.003
Hg+HBGD	-0.149 ± 0.016
Hg+BGD	2.068 ± 0.026
Hg+MBGD	2.133 ± 0.078

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 individual measurements.

is the most effective dithiocarbamate among the compounds examined in removing mercury from the body without the redistribution of mercury to other tissues, such as brain, heart, and lung, at both 30 min and 24 h after treatment with mercury.

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