Synthesis of Novel Chelating Agents and Their Effect on Cadmium Decorporation

Chao Wang, Yiou Fang, and Shiqi Peng*

School of Pharmaceutical Sciences, Beijing Medical University, Beijing 100083, PRC

Dongxin Ma and Jinyuan Zhao

Research Center of Occupational Medicine, Beijing Medical University, Beijing 100083, PRC

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A series of novel dithiocarbamates, disodium salts of *N*-glucamyl-*N*-dithiocarboxyl-amino acids, were synthesized, and their usefullness as an antagonist of cadmium intoxication was investigated. These chelating agents were found to be effective in both acute and repeated exposure cadmium poisoning. The results showed that the cadmium mobilizing properties of disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-threoninate and disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-cysteinate are clearly superior to those of sodium *N*-(4-methoxybenzyl)-D-glucamine-*N*-carbodithioate (MeOBGDTC) revealed in the experiments described here. The toxicity of these novel compounds is modest, and their effect on the concentrations of essential metal ions in the renal cortex is quite small in comparison with that of a group treated with cadmium only. The new dithiocarbomates were identified by MS, rather than by elemental analysis, as they were extremely hygroscopic.

Introduction

Of the various metals which are environmentally significant as toxic species, cadmium plays an unusual role because of the obvious accumulation in the human body. Cadmium possesses an ability to settle intracellularly by binding with a low-molecular weight protein, metallothionein (MT) (*1*). The accumulation of cadmium in the human body, mainly in liver and kidney, will lead to serious cadmium intoxication, especially the toxic renal damage. Therefore, it is important to develop effective, safe chelating agents for the therapy of cadmium intoxication.

Ethylenediaminetetraacetic acid (EDTA), 2,3-dimercaptopropanol (BAL), and penicillamine (PA) are widely used for the treatment of metal intoxication (*2*). Mercapto compounds, BAL or PA, can chelate cadmium strongly by the low-molecular weight ligands, but this kind of cadmium complex will be reabsorbed by renal tubules. It may increase the level of kidney deposition and also enhance the kidney toxicity of cadmium (*3*, *4*). On the other hand, the commonly used hydrophilic aminopolycarboxylic acids, such as EDTA and DTPA (diethylenetriaminepentaacetic acid), can form more stable complexes with cadmium, but they only have a very slight effect on the cadmium deposited in kidney, because these chelating agents are unable to enter the intracellular sites (*5*), and have a low selectivity for cadmium as metal ions (*6*).

The ability of sodium diethyldithiocarbamate (DEDTC) to act as an antagonist for acute cadmium chloride intoxication was first reported by Gale (*7*). Subsequent studies have confirmed and extended these results and showed that dithiocarbamates are capable of reacting with and mobilizing intracellular deposition of cadmium. But it enhanced the cadmium concentration in the brain, because Cd−DEDTC complexes are so lipid soluble that they can penetrate the blood−brain barrier (*8*). It was also shown that this could be prevented by appropriate substitution of polar groups in the dithiocarbamate (*9*). The studies showed that sodium *N*-methyl-D-glucamine-*N*-carbodithioate (MGDTC) possessed low toxicity and was able to remove deposited cadmium from kidney and liver in mice (*10*).

Sodium *N*-benzyl-D-glucamine-*N*-carbodithioate (BGDTC) and a series of the corresponding substituted aromatic compounds were prepared, and the results show that they were superior to MGDTC for cadmium decorporation (*11*). Unfortunately, the toxicity of these aromatic compounds was higher than that of MGDTC (*10*, *11*). It is obvious that the toxicity stems from the moiety of the aromatic structure.

In view of these reasons, we decided to prepare compounds of an analogous series which used amino acids as the source of the amine groups. The structures of the compounds prepared are shown in Figure 1. Compared with BGDTC, the glucamine moiety was retained and the benzylamine moiety was replaced by an amino acid-derived group. When cysteine is the amino acid, the chelating agent produced also contains a mercapto group, which may also act to coordinate the metal ion.

The purpose of this study was to develop novel dithiocarbamate chelating agents for in vivo cadmium mobilization. Specifically, we hoped to obtain compounds which were more effective and less toxic than those previously reported. The reactions used in the preparation of these novel chelating agents are shown in Figure 1.

Experimental Section

Chemical Synthesis. L-Amino acids were purchased from Sigma Chemical Co. Spectroscopic data and physical and

* To whom correspondence should be addressed.

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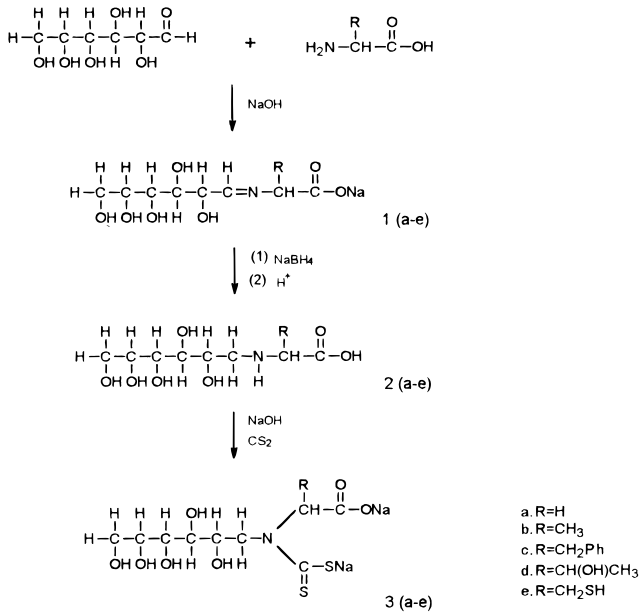

Figure 1. Synthetic scheme used for the preparation of the novel chelating agents.

analytical data were obtained on the instruments listed: IR, Perkin-Elmer 983; NMR, Bruker AM-500; MS, VG-ZAB-MS; elemental analysis, PE-2400; molecular rotation, Polartronic-D; and HPLC, Waters 510.

Sodium *N*-(2,3,4,5,6-Pentahydroxylhexylidene)glycinate (1a). D-(+)-Glucose (5.94 g, 30 mmol), glycine (2.25 g, 30 mmol), and NaOH (1.2 g, 30 mmol) were allowed to react in water (3 mL) at 60 °C for 5 h to give a gel: FAB/MS (*m*/*e*) 260 $[M + H]^+$.

***N*-(2,3,4,5,6-Pentahydroxylhexyl)glycine (2a).** The crude imine (**1a**) was reduced to amine by $NaBH_4$ (3.0 g) at room temperature for 120 h. The reaction mixture was acidified by HCl (6 N) to pH 2 at 0 °C. This solution was concentrated to a syrup. Then, 30 mL of methanol was added, and the inorganic salts were separated by filtration. The filtrate can be recrystallized several times in H_2O/EtOH to give a white crystalline solid (yield of 60%, two steps): mp 182−183 °C; FAB/MS (*m*/*e*) 240 $[M + H]^+$; ^{1}H NMR (D_2O) δ 3.52 (m, 2H, 6-H), 3.62 (m, 1H, 5-H), 3.68 (dd, J = 11.0, 3.5 Hz, 1H, 4-H), 3.70 (t, J = 2.0 Hz, 1H, 3-H), 3.99 (m, 1H, 2-H), 3.07 (dd, J = 13.5, 9.5 Hz, 1H, 1-H), 3.16 (dd, J = 13.0, 3.0 Hz, 1H, 1-H), 3.54 (d, J = 2.0 Hz, 2H, Gly-CH_2); IR (KBr) 3222, 3020, 2926, 1617, 1563, 1467, 1377, 1245, 1122, 1092, 1059, 1037 cm^{-1}. Anal. Calcd for $C_8H_{17}N_1O_7$: C, 40.17; H, 7.16; N, 5.86. Found: C, 40.37; H, 6.90; N, 5.64.

Disodium *N*-(2,3,4,5,6-Pentahydroxylhexyl)-*N*-dithiocarbamate-glycinate (3a). 2a (3.0 g, 12.6 mmol), NaOH (0.5 g, 12.6 mmol), and 10 mL of water were stirred under argon at 0 °C. CS_2 (1.9 g, 25.0 mmol) in dioxane (10 mL) and NaOH (0.5 g, 12.6 mmol) in water (5 mL) were added dropwise to the clear reaction mixture, while it was being stirred at 0 °C over the course of 1 h, and then further stirred overnight at room temprature. The mixture was put under reduced pressure to remove excess CS_2, and then the resulting solution was frozen and lyophilized to obtain the crude product. It was recrystallized from H_2O/EtOH to give a yellow solid (yield of 89%): mp 100−102 °C dec; FAB/MS (*m*/*e*) 358 $[M - H]^-$; IR (KBr) 3408, 2960, 1588, 1460, 1377, 1209, 1169, 1055 cm^{-1}.

Compounds **1b**−**e**, **2b**−**e**, and **3b**−**e** were prepared in a manner similar to that described above, and the corresponding yield, physical data, and analytical results for each are shown as follows.

Sodium *N*-(2,3,4,5,6-pentahydroxylhexylidene)-L-alaninate (1b): FAB/MS (*m*/*e*) 274 $[M + H]^+$.

Sodium *N*-(2,3,4,5,6-pentahydroxylhexylidene)-L-phenylalaninate (1c): FAB/MS (*m*/*e*) 350 $[M + H]^+$.

Table 1. Liver Cadmium Levels after Treatment with Chelating Agents during Acute Intoxication[a]

	4 h	24 h	48 h
Cd only	24.4 ± 0.69	20.4 ± 0.48	20.1 ± 0.59
Cd and MeOBGDTC	24.4 ± 3.82	15.4 ± 1.79[b,d]	13.5 ± 0.86[b,f]
Cd and **3a**	24.1 ± 1.36	14.8 ± 0.52[b,f]	15.3 ± 0.55[b,f]
Cd and **3b**	21.9 ± 1.23	17.9 ± 0.90[b,d]	14.6 ± 0.99[b,e]
Cd and **3c**	18.7 ± 4.56	16.6 ± 1.03[b,e]	14.3 ± 1.02[b,e]
Cd and **3d**	28.5 ± 0.92	22.1 ± 0.58	9.9 ± 0.38[b,c,e,f]
Cd and **3e**	13.0 ± 1.42[b−d,f]	15.8 ± 0.56[b,f]	11.0 ± 0.38[b−d,f]

[a] Micrograms per gram of tissue weight (X ± SE) where $n = 5$. [b] Significantly less than that with Cd only. [c] Significantly less than that with MeOBGDTC. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P < 0.001$.

Table 2. Renal Cortical Cadmium Levels after Treatment with Chelating Agents during Acute Intoxication[a]

	4 h	24 h	48 h
Cd only	25.5 ± 0.66	20.6 ± 0.66	18.4 ± 1.32
Cd and MeOBGDTC	14.6 ± 0.34[b,f]	15.0 ± 1.23[b,e]	13.1 ± 0.71[b,e]
Cd and **3a**	17.4 ± 0.62[b,f]	17.6 ± 0.67[b,d]	16.6 ± 0.64
Cd and **3b**	20.2 ± 1.01[b,e]	21.2 ± 0.85	20.9 ± 0.62
Cd and **3c**	15.8 ± 0.61[b,f]	12.9 ± 0.47[b,f]	10.8 ± 0.42[b−d,f]
Cd and **3d**	12.1 ± 0.68[b,c,e,f]	15.5 ± 0.61[b,f]	12.2 ± 0.47[b,e]
Cd and **3e**	9.8 ± 0.90[b,c,e,f]	11.2 ± 0.94[b−d,f]	11.7 ± 0.06[b,f]

[a] Micrograms per gram of tissue weight (X ± SE) where $n = 5$. [b] Significantly less than that with Cd only. [c] Significantly less than that with MeOBGDTC. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P < 0.001$.

Sodium *N*-(2,3,4,5,6-pentahydroxylhexylidene)-L-threoninate (1d): FAB/MS (*m*/*e*) 304 $[M + H]^+$.

Sodium *N*-(2,3,4,5,6-pentahydroxylhexylidene)-L-cysteinate (1e): FAB/MS (*m*/*e*) 306 $[M + H]^+$.

***N*-(2,3,4,5,6-Pentahydroxylhexyl)-L-alanine (2b):** yield 57% (two steps); mp 201−203 °C; FAB/MS (*m*/*e*) 254 $[M + H]^+$; ^{1}H NMR (D_2O) δ 3.51 (m, 2H, 6-H), 3.62 (m, 1H, 5-H), 3.68 (dd, J = 10.5, 3.0 Hz, 1H, 4-H), 3.69 (t, J = 2.6 Hz, 1H, 3-H), 3.96 (m, 1H, 2-H), 3.03 (dd, J = 12.8, 9.7 Hz, 1H, 1-H), 3.14 (dd, J = 12.8, 3.2 Hz, 1H, 1-H), 3.61 (q, J = 3.1 Hz, 1H, Ala-CH), 1.38 (d, 3H, CH_3); IR (KBr) 3410, 3270, 2972, 2940, 1903, 1621, 1585, 1479, 1421, 1396, 1367, 1287, 1267, 1146, 1131, 1014, 1003 cm^{-1}. Anal. Calcd for $C_9H_{19}N_1O_7$: C, 42.68; H, 7.56; N, 5.53. Found: C, 42.39; H, 7.33; N, 5.55.

***N*-(2,3,4,5,6-Pentahydroxylhexyl)-L-phenylalanine (2c):** yield 38% (two steps); mp 213−215 °C; FAB/MS (*m*/*e*) 330 $[M + H]^+$; ^{1}H NMR (D_2O) δ 3.48 (m, 2H, 6-H), 3.58 (m, 1H, 5-H), 3.65 (dd, J = 11.2, 3.0 Hz, 1H, 4-H), 3.66 (t, J = 2.2 Hz, 1H, 3-H), 3.91 (m, 1H, 2-H), 2.97 (dd, J = 12.5, 9.5 Hz, 1H, 1-H), 3.05 (dd, J = 12.5, 3.2 Hz, 1H, 1-H), 3.82 (t, J = 6.0 Hz, 1H, Phe-CH), 3.10 (d, J = 5.5 Hz, 2H, CH_2), 7.15−7.30 (5H, Ar-H); IR (KBr) 3361, 3105, 3025, 2922, 2655, 1617, 1491, 1430, 1371, 1297, 1249, 1218, 1122, 1081, 1043 cm^{-1}. Anal. Calcd for $C_{15}H_{23}N_1O_7 \cdot {}^1/_2HCl$: C, 51.83; H, 6.82; N, 4.03. Found: C, 52.20; H, 6.97; N, 3.99.

***N*-(2,3,4,5,6-Pentahydroxylhexyl)-L-threonine (2d):** yield 51% (two steps); mp 219−221 °C; FAB/MS (*m*/*e*) 284 $[M + H]^+$; ^{1}H NMR (D_2O) δ 3.44 (m, 2H, 6-H), 3.55 (m, 1H, 5-H), 3.61 (dd, J = 11.7, 2.8 Hz, 1H, 4-H), 3.63 (t, J = 2.1 Hz, 1H, 3-H), 3.94 (m, 1H, 2-H), 2.99 (dd, J = 13.7, 10.0 Hz, 1H, 1-H), 3.09 (dd, J = 12.9, 3.2 Hz, 1H, 1-H), 3.30 (d, J = 7.7 Hz, 1H, Thr-CH), 3.88 (m, 1H, Thr-CH), 1.14 (d, J = 6.5 Hz, 3H, CH_3); IR (KBr) 3383, 3250, 2982, 2944, 1570, 1446, 1391, 1325, 1303, 1208, 1133, 1109, 1057, 1035 cm^{-1}. Anal. Calcd for $C_{10}H_{21}N_1O_8$: C, 42.38; H, 7.47; N, 4.95. Found: C, 42.35; H, 7.05; N, 4.74.

***N*-(2,3,4,5,6-Pentahydroxylhexyl)-L-cysteine (2e):** yield 35% (two steps); mp 197−199 °C; FAB/MS (*m*/*e*) 286 $[M + H]^+$; ^{1}H NMR (D_2O) δ 3.41 (m, 2H, 6-H), 3.49 (m, 1H, 5-H), 3.55 (dd, J = 10.5, 3.6 Hz, 1H, 4-H), 3.58 (t, J = 2.5 Hz, 1H, 3-H), 3.90 (m, 1H, 2-H), 2.95 (dd, J = 12.9, 8.4 Hz, 1H, 1-H), 3.09 (dd, J = 12.8, 3.6 Hz, 1H, 1-H), 3.72 (t, J = 5.1 Hz, 1H, Cys-CH), 2.86 (dd, J = 9.0, 4.8 Hz, 2H, Cys-CH_2); IR (KBr) 3271, 2932, 1603,

Table 3. Blood Cadmium Levels after Treatment with Chelating Agents during Acute Intoxication[a]

	4 h	24 h	48 h
Cd only	0.06 ± 0.02	<0.01 ± 0.00	<0.01 ± 0.00
Cd and MeOBGDTC	0.18 ± 0.03[b,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3a**	0.91 ± 0.10[b,c,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3b**	0.28 ± 0.04[b,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3c**	0.13 ± 0.03[b,d]	0.01 ± 0.00	0.05 ± 0.01
Cd and **3d**	0.88 ± 0.04[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3e**	0.49 ± 0.02[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00

[a] Micrograms per milliliter (X ± SE) where $n = 5$. [b] Significantly more than that with Cd only. [c] Significantly more than that with MeOBGDTC. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P < 0.001$.

Table 4. Urinary Cadmium Levels after Treatment with Chelating Agents during Acute Intoxication[a]

	4 h	24 h	48 h
Cd only	0.03 ± 0.004	<0.01 ± 0.00	<0.01 ± 0.00
Cd and MeOBGDTC	0.10 ± 0.001[b,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3a**	1.68 ± 0.37[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3b**	0.59 ± 0.07[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3c**	3.60 ± 0.41[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3d**	1.82 ± 0.21[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00
Cd and **3e**	6.25 ± 0.26[b,c,e,f]	<0.01 ± 0.00	<0.01 ± 0.00

[a] Micrograms per milliliter (X ± SE) where $n = 5$. [b] Significantly more than that with Cd only. [c] Significantly more than that with MeOBGDTC. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P < 0.001$.

Table 5. Cadmium Levels following Chelate Treatment of Mice in Repeated Exposure Mode[a]

	liver	kidney
Cd only	33.4 ± 0.30	20.1 ± 0.21
Cd and MeOBGDTC	15.6 ± 0.22[b,f]	5.6 ± 0.35[b,f]
Cd and **3a**	19.3 ± 0.25[b,f]	8.2 ± 0.43[b,f]
Cd and **3b**	18.9 ± 1.07[b,f]	10.1 ± 0.27[b,f]
Cd and **3c**	16.6 ± 0.48[b,f]	5.0 ± 0.89[b,f]
Cd and **3d**	13.3 ± 0.24[b−d,f]	8.50 ± 0.069[b,f]
Cd and **3e**	14.6 ± 0.38[b−d,f]	4.2 ± 0.23[b,c,e,f]

[a] Micrograms per gram of tissue weight (X ± SE) where $n = 8$. [b] Significantly less than that with Cd only. [c] Significantly less than that with MeOBGDTC. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P < 0.001$.

1565, 1414, 1388, 1350, 1290, 1131, 1082, 1036 cm^{-1}. Anal. Calcd for $C_9H_{19}N_1O_7S_1$: C, 37.89; H, 6.71; N, 4.91. Found: C, 37.69; H, 6.43; N, 4.91.

Disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-alaninate (3b): yield 89%; mp 103−105 °C dec; FAB/MS (*m*/*e*) 372 $[M - H]^-$, 350 $[M - Na]^-$; IR (KBr) 3378, 2923, 1584, 1389, 1254, 1171, 1076 cm^{-1}.

Disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-phenylalaninate (3c): yield 80%; mp 145−147 °C dec; FAB/MS (*m*/*e*) 448 $[M - H]^-$, 426 $[M - Na]^-$; IR (KBr) 3350, 2925, 1588, 1449, 1381, 1225, 1160 cm^{-1}.

Disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-threoninate (3d): yield 87%; mp 78−81 °C dec; FAB/MS (*m*/*e*) 402 $[M - H]^-$, 380 $[M - Na]^-$; IR (KBr) 3334, 2923, 1588, 1388, 1227, 1079 cm^{-1}.

Disodium *N*-(2,3,4,5,6-pentahydroxylhexyl)-*N*-dithiocarbamate-L-cysteinate (3e): yield 62%; mp 104−106 °C dec; FAB/MS (*m*/*e*) 404 $[M - H]^-$; IR (KBr) 3368, 2923, 1600, 1386, 1224, 1178, 1083 cm^{-1}.

The preparations of MeOBGDTC were made following the methods of Jones and co-workers (*11*). The product was characterized by negative FAB/MS and elemental analysis.

Animal Studies. Acute studies. Adult male Wistar rats weighing 250 ± 50 g were used for experiments. Each of them was given a single 15 μmol/kg injection (ip) of cadmium chloride mixed with mercaptoethanol (ME) at 300 μmol/kg in a final volume of 2.5 mL of saline. Two hours later, they were further treated (ip) with chelating agents, 0.25 mmol/kg in a final volume of 2.5 mL of water. The cadmium-only group was given a 0.9% saline injection (ip). Biosamples were collected 4, 24, and 48 h after cadmium injection. Rats were killed under pentobarbital anesthesia (ip); blood (by cardiac puncture), liver, and kidney samples were then obtained, and urine samples were periodically collected from individual metabolic cages.

Repeated Exposure Studies. Male ICR mice weighing 25 ± 2 g were loaded with cadmium via a series of injections (ip) by administering four 3 mg injections of $CdCl_2 \cdot 2.5H_2O$/kg in 0.5 mL of 0.9% saline on four consecutive days. After a 3 day interval, the animals were randomly divided into groups of seven each. One group served as the cadmium-only group and was given 0.9% saline injections (ip) instead of chelating agent. Each member of the other groups was given an injection (ip) of one of the chelating agents at a level of 1.0 mmol/kg in 0.5 mL of water each day for five consecutive days. Two days after the last injection of chelating agent, all the animals were sacrificed by cervical dislocation and the livers and kidneys immediately excised.

Biosamples were digested in H_2SO_4, $HClO_4$, and HNO_3 (1:1:3) on a heating block, dried at 80 °C, redissolved in 1% nitric acid, and analyzed for cadmium content using a HITACHI 180-80 atomic absorption spectrometer in the flame mode. The variety of trace metals were determined by synchrotron X-ray fluorescence. The LD_{50} values were determined by single injection (ip) in mice.

All animals were kept in an AALAC approved animal care facility during the course of the experiments and were provided with free access to food and water. The statistical analysis of the data was carried out by using standard analysis of variance methods.

Results and Discussion

The results of the acute studies are summarized in Table 1−4. The data in Tables 1 and 2 indicate that all of the compounds were reasonably effective antagonists under this experimental condition and significantly reduced the level of cadmium in liver and kidney in comparison with the control (Cd only) in most situations. Compound **3d** in 48 h and compound **3e** in 4 or 48 h are clearly superior to MeOBGDTC with respect to liver

Table 6. Influence of Chelating Agents on the Renal Cortex Concentration of Essential Metals in Rats[a]

		Zn	Cu	Fe	Mn	Ca	Rb
Cd only	4 h	21.8 ± 1.24	5.4 ± 0.25	50.2 ± 1.93	1.2 ± 0.20	62.4 ± 3.93	9.6 ± 0.60
	24 h	21.8 ± 3.27	6.6 ± 0.51	45.2 ± 3.60	1.0 ± 0.00	126.6 ± 47.3	8.0 ± 0.71
Cd and MeOBGDTC	4 h	22.0 ± 0.32	5.5 ± 0.58	45.4 ± 5.03	1.0 ± 0.00	51.8 ± 3.57	10.0 ± 0.55
	24 h	28.4 ± 1.03	7.0 ± 0.97	54.4 ± 5.84	1.2 ± 0.20	77.4 ± 18.9	12.4 ± 0.68
Cd and **3c**	4 h	21.0 ± 1.00	6.0 ± 0.55	47.2 ± 3.26	1.0 ± 0.00	53.8 ± 7.59	14.4 ± 0.75
	24 h	22.6 ± 0.68	6.6 ± 0.25	44.4 ± 2.21	1.0 ± 0.00	61.2 ± 4.22	8.4 ± 0.40
Cd and **3d**	4 h	22.0 ± 0.55	10.4 ± 0.40	37.8 ± 0.37	1.2 ± 0.20	66.2 ± 6.14	16.6 ± 0.25
	24 h	19.0 ± 0.84	5.4 ± 0.25	56.0 ± 1.45	1.2 ± 0.20	62.0 ± 2.72	8.2 ± 0.37
Cd and **3e**	4 h	19.8 ± 0.58	5.4 ± 0.25	51.4 ± 5.54	0.8 ± 0.20	65.0 ± 4.18	9.0 ± 0.71
	24 h	23.8 ± 0.49	5.2 ± 0.37	61.4 ± 3.06	1.2 ± 0.20	79.6 ± 7.36	9.0 ± 0.00

[a] Micrograms per gram of tissue weight (X ± SE) where $n = 5$.

cadmium decorporation. Compound **3c** in 48 h, compound **3d** in 4 h, and compound **3e** in 4 or 24 h are clearly superior to MeOBGDTC with respect to kidney cadmium decorporation.

The data in Tables 3 and 4 are the concentrations of cadmium in blood and urine. As illustrated in Table 3, the concentrations of cadmium in blood were increased 2 h after injection of the chelators, which presumably reflects in part mobilization of cadmium from tissues. The effects of all chelating agents on the urinary excretion of cadmium in rats are shown in Table 4. It was a gross increase in the level of urinary excretion of cadmium with use of chelators in the 4 h group. The increased level of urinary excretion of cadmium by all of the new compounds is significantly more then that by MeOBGDTC. These results suggest that such structures of disodium salts with good water solubility can make cadmium complexes more easily excreted in urine.

The all new dithiocarbamates also proved to be efficacious in repeated exposure cadmium intoxication as shown in Table 5. All of the compounds were found to be able to remove cadmium from the liver and kidney. Compounds **3d** and **3e** are superior to MeOBGDTC in removing cadmium from the liver, and **3e** is clearly superior in removing cadmium from the kidney. Within 24 h of the injection of cadmium into a rodent, the majority of the element is present in intracellular sites (*12*), and at the end of 3 days, most of this intracellular cadmium is present as the complex with MT (*13*). So the results in repeated exposure studies indicate that these compounds are able to remove cadmium from its deposits in both the liver and kidney.

The results in Table 6 showed that the concentration of Zn, Cu, Fe, Mn, Ca, and Rb in the kidney of rats after treatment with MeOBGDTC and **3c**−**e** exhibited little change as compared with that of the group treated with cadmium only. The data in Table 6 were recorded for cadmium-intoxicated animals which have been treated with cadmium, described as acute studies.

A problem in this experiment is these new chelating agents were extremely hygroscopic in air. Therefore, the repeatability of elemental analysis was very low. Fortunately, the structures of these compounds were identified by negative FAB/MS, and the purity was ascertained by high-performance liquid chromatography, with a purity of >98%.

Compound **2e** or **3e** must be isolated from air to prevent oxidation while it is being prepared because the mercapto group can transform into disulfide easily under alkaline conditions.

The experimental model with a single injection (ip) of $CdCl_2$ and ME in rats is simple, rapid, and useful for the screening of the new chelators, and it can clearly reflect cadmium-induced renal damage within 24 h of cadmium exposure (*14*).

Singh et al. (*15*) indicated not only that the structure of the chelator must have the proper type of chelating group but also that the presence of appropriate additional groups in the molecule is essential for an appreciable level of efficacy. In the structures of these new compounds presented here, the additional groups come from the side chain of the amino acids, and according to the theory of coordination chemistry, some heteroatoms can participate in coordination with heavy metals to form stable complexes. That probably is the reason why the effects of **3d** and **3e** are more beneficial than the effects of **3a** and **3b** on cadmium excretion.

The further study on representative compounds showed that the LD_{50} of **3d** was 10.5 mmol/kg (ip) and that of **3e** 8.6 mmol/kg (ip). The LD_{50} values for **3d** and **3e** are greater than that of PA [2.53 mmol/kg (ip)] and DEDTC [8.35 mmol/kg (ip)] (*16*).

Thus, these novel dithiocarbamates deserve further consideration as members of a small but growing group of compounds which may eventually be proved to be suitable antidotes for cadmium intoxication.

References

(1) Webb, M. (1986) Role of metallothionein in cadmium metabolism. In *Cadmium* (Foulkes, E. C., Ed.) pp 281−337, Springer-Verlag, Berlin.
(2) Andersen, O. (1989) Oral cadmium exposure in mice: Toxicokinetics and efficiency of chelating agents, *Toxicology* **20**, 83−112.
(3) Dalhamn, T., and Friberg, F. (1955) Dimercaprol (2,3-dimercaptopropanol) in chronic cadmium poisoning. *Acta Pharmacol.* **11**, 68−78.
(4) Lyle, W. H., Green, J. N., Gore, V., and Vidler, J. (1968) Enhancement of cadmium nephrotoxicity by penicillamine in the rat. *Postgrad. Med. J., Suppl.*, 18−25.
(5) Waalkes, M. P., Watkins, J. B., and Klaassen, C. D. (1983) Minimal role of metallothionein in decreased chelator efficacy for cadmium. *Toxicol. Appl. Pharmacol.* **56**, 392−398.
(6) Anderson, O. (1984) Chelation of cadmium. *Environ. Health Perspect.* **54**, 249−257.
(7) Gale, G. R., Smith, A. B., and Walker, E. M. (1981) Diethyldithiocarbamate in the treatment of acute cadmium poisoning. *Ann. Clin. Lab. Med.* **11**, 476−483.
(8) Jones, S. G., Basinger, M. A., Jones, M. M., and Gibbs, S. J. (1982) Polarity and antidotal efficacy of dithiocarbamates in acute cadmium intoxication. *Res. Commun. Chem. Pathol. Pharmacol.* **40**, 155−164.
(9) Jones, S. G., and Jones, M. M. (1984) Structure-activity relationships among dithiocarbamate antidotes for acute cadmium chloride intoxication. *Environ. Health Perspect.* **54**, 285−290.
(10) Shinobu, L. A., Jones, S. G., and Jones, M. M. (1984) Sodium *N*-methyl-D-glucamine dithiocarbamate and cadmium intoxication. *Acta Pharmacol. Toxicol.* **54**, 189−194.
(11) Jones, S. G., Singh, P. K., and Jones, M. M. (1988) Use of the Topliss scheme for the design of more effective chelating agents for cadmium decorporation. *Chem. Res. Toxicol.* **1**, 234−237.
(12) Shaikh, Z. A., and Lucis, O. J. (1972) Biological differences in cadmium and zinc turnover. *Arch. Environ. Health* **24,** 410−418.
(13) Cantilena, L. R., Jr., and Klaassen, C. D. (1982) Decreased effectiveness of chelation therapy with time after acute cadmium poisoning. *Toxicol. Appl. Pharmacol.* **63**, 173−180.
(14) Zhao, J. Y., Foulkes, E. C., and Jones, M. M. (1990) Delayed nephrotoxic effects of cadmium and their reversibility by chelation. *Toxicology* **64**, 235−243.
(15) Singh, P. K., Jones, M. M., Jones, S. G., Gale, G. R., Atkins, L. M., Smith, A. B., and Bulman, R. A. (1989) Effect of chelating agent structure on the mobilization of cadmium from intracellular deposits. *J. Toxicol. Environ. Health* **28**, 501−518.
(16) Cantilena, L. R., Jr., and Klaassen, C. D. (1981) Comparison of the effectiveness of several chelators after single administration on the toxicity, excretion and distribution of cadmium. *Toxicol. Appl. Pharmacol.* **58**, 452−460.

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