Comparative Effects of Three Dialkyldithiocarbamates on Acute Toxicity, Organ Distribution, and Excretion of Cadmium*

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ABSTRACT

Diethylldithiocarbamate (DeDTC), dimethyldithiocarbamate (DmDTC), and diisopropyldithiocarbamate (DiDTC) were compared at equimolar doses to determine their relative efficacies in antagonizing the acute toxicity of a >LD_{100} dose of cadmium (Cd) and in promoting the mobilization, redistribution, and excretion of metallothionein-bound {^{109}Cd} in mice receiving a <LD_{10} dose of Cd prior to initiation of treatment. The DeDTC was an effective antidote for a >LD_{100} dose of Cd if given 30 min to two hrs after Cd, but was totally ineffective if given 30 min prior to Cd. In contrast, DmDTC was completely protective at all times studied. When DiDTC was given prior to Cd, mice died sooner than those which received Cd alone, and DiDTC was not an effective antagonist when given after Cd. Only two of eight mice survived, and these had received DiDTC two hrs after Cd. The gross pathology of organs of mice which were treated with each analog and which survived 30 days after Cd administration was of the order DeDTC < DmDTC << DiDTC. There was a marked similarity in the actions of DeDTC and DmDTC in promoting mobilization, redistribution, and excretion of metallothionein-bound Cd; both analogs extensively reduced the hepatic, renal, intestinal, and splenic Cd burdens, but enhanced the Cd load of lung, testes, heart, and brain. The rate of fecal Cd excretion was substantially the same following treatment with both compounds and markedly exceeded the control excretion rate. In contrast, DiDTC reduced the hepatic, renal, intestinal, and splenic Cd levels, but did not promote accumulation in the lung. In addition, DiDTC

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reduced rather than increased the testicular Cd burden. The reduction by DiDTC of hepatic, renal, intestinal, and splenic levels was not accompanied by a greatly enhanced rate of Cd excretion. Evaluation of Cd content of additional tissues revealed that DiDTC caused a marked Cd redistribution to striated muscle; Cd content of this tissue from DiDTC-treated mice was about 14 times higher than that of striated muscle from control mice which received Cd only. Upon dissection of mice following seven and 13 injections of each compound, with treatment begun 15 days after administration of a <LD100 dose of Cd, none of the gross pathological changes were observed which ensued after a single injection of each following administration of the >LD100 Cd dose used in the acute toxicity experiments.

Introduction

The high degree of success achieved by Sunderman et al in treating experimental and clinical nickel poisoning with diethyldithiocarbamate (DeDTC)\textsuperscript{9,10,11,12,13,14} prompted an earlier study of this compound in experimental acute cadmium (Cd) poisoning in mice.\textsuperscript{7} Substantial antidotal effectiveness was noted in mice which received a >LD100 dose of Cd (10.0 mg per kg of CdCl\textsubscript{2} • 2.5H\textsubscript{2}O; = 4.92 mg per kg of Cd\textsuperscript{++}). In marked contrast to the action of certain other chelators,\textsuperscript{4} DeDTC was more effective when treatment was delayed for 30 min to five hrs than when it was given prior to or immediately after Cd.\textsuperscript{7} Subsequent work revealed that DeDTC was extremely effective in mobilizing Cd from its metallothionein-bound sites in liver, kidney, and spleen, but promoted a marked accumulation in brain and a lesser extent in lung, testes, and heart.\textsuperscript{5} While diethylenetriaminepentaacetate and dimercapto-succinate are highly effective antidotes for acute Cd intoxication when given simultaneously with or shortly after Cd,\textsuperscript{1,3} the former is only minimally effective in promoting mobilization and excretion of metallothionein-bound renal Cd, and the latter was totally ineffective under the experimental conditions used.\textsuperscript{6}

West and Sunderman found that certain other alkylidithiocarbamates conferred 100 percent protection against an otherwise lethal concentration of Ni-(CO)\textsubscript{4}.\textsuperscript{14} Those with efficacies approximating that of DeDTC were dimethyl-dithiocarbamate (DmDTC) and diisopropylidithiocarbamate (DiDTC), and the acute toxicity of each analog was similar to that of DeDTC.

The current study compares the effects of DeDTC, DmDTC, and DiDTC on acute toxicity of Cd as well as effects on distribution and excretion of Cd using the virtually steady-state model in which Cd is maximally bound to metallothionein.\textsuperscript{5,6}

Materials and Methods

Male mice of the (DBA/2 × C57BL/6)F\textsubscript{1} strain* (BDF\textsubscript{1}) were used in all experiments. The CdCl\textsubscript{2} • 2.5 H\textsubscript{2}O for acute toxicity studies and as carrier in chronic \textsuperscript{109}Cd loading experiments was certified A.C.S. grade.\textsuperscript{†} The \textsuperscript{109}Cd, as \textsuperscript{109}CdCl\textsubscript{2} in aqueous solution, was accelerator-produced and carrier-free.\textsuperscript{‡} The DeDTC was obtained as the sodium salt, trihydrate.\textsuperscript{†}

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† Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, NJ.
‡ New England Nuclear, Boston, MA.
The DmDTC was ordered from the same supplier (in whose catalog it was described as reagent grade), but on arrival bore the label of a different manufacturer. Karl-Fisher water analyses, however, showed that this compound contained 40.34 percent water. The DiDTC was synthesized as described by Kopping and van der Kerk. Upon heating all three compounds to 65° for 24 hours in vacuo, water analyses, as well as carbon, hydrogen, and nitrogen analyses of DiDTC, were consistent with one water of hydration for both DmDTC and DiDTC, and the DeDTC contained <2.0 percent water. All dithiocarbamates were stored in vacuo, were weighed rapidly, and the formula weight of each appropriate hydration state was used in calculating equimolar doses. The dithiocarbamates were administered in one percent NaHCO₃ solution, 1.0 ml per 30 g of body weight; Cd was administered in 0.9 percent NaCl solution.

To compare antidotal efficacies in acute Cd poisoning, groups of eight mice (24.9 ± 0.8 g) were given an i.p. >LD₁₀₀ dose of 10.0 mg per kg of CdCl₂ · 2.5 H₂O preceded by or followed, at various intervals, by i.p. administration of 2.22 mmole per kg of DeDTC, DmDTC, or DiDTC. Two groups of eight control mice received only the Cd solution. Mortality was recorded daily, and all surviving animals at 30 days were sacrificed and dissected for gross observations of selected organs.

For studies of mobilization and excretion of metallothionein-bound Cd, 80 mice with a mean weight of 24.0 ± 1.7 g were divided into four groups of 20 each; there was no significant difference in mean body weight between the groups. Each mouse received an i.p. injection of 0.03 mg of CdCl₂ · 2.5 H₂O containing 1.0 μCi ¹⁰⁹Cd. This dose is well below the LD₅₀ dose. On day 15 after Cd injections, mice were placed on a regimen of treatment with each compound given at 2.22 mmole per kg thrice weekly. One group of 20 served as controls and received the appropriate volume of one percent NaHCO₃ solution. One day after the seventh injection of each dithiocarbamate, mice were subjected to whole-body gamma counting as previously described. On the following day, six mice from each group were sacrificed and selected organs were removed for determination of radioactivity in a gamma well counter. The remaining mice received an additional six injections on the same schedule as before, after which whole-body gamma counting and measurements of individual organ radioactivity were done.

To determine the effect of each dithiocarbamate on rate and route of Cd excretion, six mice were placed into each of four plastic metabolism cages 15 days after receiving Cd with ¹⁰⁹Cd. Mice in each group were then given three daily injections of each compound at 2.22 mmole per kg, and six mice served as controls. Twenty-four hours after each injection, total feces and urine were collected separately and radioactivity determined. The total cpm of each, after dividing by 6 (the number of mice in each cage), was used to calculate the amount excreted daily per mouse expressed as a function of the total Cd administered. Statistical evaluations were done with a microcomputer with appropriate software programs.

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†† Beckman Instruments, Inc., Fullerton, CA; Bio-gamma model I.
** Plas-Labs, Lansing, MI; model no. XPL-900-RCS.
†† Tandy Corp., Fort Worth, TX; TRS-80 Model I Level II 16K microcomputer with Advanced Statistical Software Programs (Catalog no. 26-1705).
Results

CHEMICAL STUDIES

To assess the relative reactivity of each analog with various cations, 1.0 ml of a 2 × 10⁻³ M aqueous solution of each was added to an equal volume of a 10⁻³ M aqueous solution of each of various soluble metal salts; the associated anion of each was either sulfate, chloride, acetate, or nitrate, as described in an earlier report.⁷ The reactions were qualitatively quite similar, with only minimal quantitative variances, indicating only slight differences in in vitro reactivity with each of the cations tested. As was found with DeDTC,⁷ neither DmDTC nor DiDTC caused any aberrations of the absorption spectrum of oxyhemoglobin from osmotically-lysed human erythrocytes.

Elemental analyses of the insoluble complex of DeDTC with Cd in an earlier study⁷ were consistent with a 2:1 chelate: Cd complex. To determine if a similar complex occurs with DmDTC and DiDTC when reacted with Cd, an aqueous solution of each chelator was added to an aqueous solution of CdCl₂ · 2.5 H₂O in a 2.2:1 chelator: Cd molar ratio. After stirring for 30 min, the insoluble precipitate was collected by vacuum filtration, washed extensively with deionized water, and dried at 65° in vacuo for 24 hr. Elemental analyses were consistent with a structure of each similar to that found earlier with DeDTC,⁷ or a 2:1 chelator: Cd complex. Neither of the complexes was soluble in water, 1.0 N HCl, 1.0 N NaOH, or carbon tetrachloride.

ACUTE TOXICITY STUDIES

The effects of an equimolar dose of each chelator, when given prior to or at various intervals after a >LD₁₀₀ i.p. dose of Cd, are shown in table I. There were considerable differences in the antidotal efficacies among the three analogs. Diethylthiocarbamate was most effective when treatment was delayed for 30 min or longer. When given 30 min prior to Cd, all mice succumbed at approximately the same rate as those which received Cd alone. In contrast, DmDTC was totally protective when given 30 min before and up to two hrs following Cd. The action of DiDTC was markedly different from the other two analogs. When given 30 min prior to Cd, mortality was 100 percent within 24 hrs; of those mice which received Cd only, 11 of 16 (69 percent) survived a minimum of 24 hrs, and 100 percent mortality was attained only after three days. Diisopropylthiocarbamate given up to one hr after Cd showed no protective effect, and only 25 percent of the mice survived for 30 days when treatment was delayed for two hrs. All mice which received DiDTC displayed a transient hyperexcitability shortly after injection, but this was not observed with the remaining two analogs.

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<th>Treatment</th>
<th>Survivors on Day</th>
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<td>1</td>
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<tr>
<td>Cd only*</td>
<td>11/16</td>
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<tr>
<td>Cd + DeDTC† (-30 min)‡</td>
<td>6/8</td>
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<tr>
<td>Cd + DmDTC† (-30 min)</td>
<td>8/8</td>
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<td>Cd + DiDTC† (-30 min)</td>
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*10 mg per kg of CdCl₂ · 2.5 H₂O = 4.92 mg per kg of Cd⁺⁺ on day 0.
‡2.22 mmole per kg.
†Indicates time of injection of each dithiocarbamate before (-) or after (+) injection of cadmium.
After 30 days, surviving mice were sacrificed and examined for evidence of gross pathology. Of the 24 surviving mice which received DeDTC, there were few remarkable changes noted. One of eight which received the chelator 30 min after Cd had pale and moderately atrophic kidneys and liver; two of eight which received it two hrs after Cd had pale kidneys, and in one of those there was also slight renal atrophy. One mouse of eight which received DeDTC one hr after Cd showed some peritoneal adhesions.

The most consistent pathological finding in all mice given DmDTC 30 min prior to Cd was the presence of severe peritoneal adhesions, often matting adjacent structures to the extent that separation was difficult. Kidneys were atrophic in five of eight mice, and most were pale. Six of eight livers were atrophic with change of shape secondary to adhesions of adjacent structures. These gross pathological changes were either absent or markedly reduced in all 24 mice which received DmDTC 30 min, one hr, or two hrs after Cd.

The two surviving mice which received DiDTC two hrs after Cd had massive, severe intraabdominal adhesions which made separation of organs virtually impossible. Livers were atrophic, and kidneys were atrophic and pale. The degree of gross pathology in these two mice markedly exceeded any of the changes seen in the mice which received DmDTC.

**DISTRIBUTION AND EXCRETION STUDIES**

The effect of seven injections of each analog on the Cd content of selected organs of mice which had received Cd 15 days before beginning treatment is shown in figure 1. As was noted in the acute toxicity study, all mice which received DiDTC became agitated and hyperexcitable shortly after each injection. This period of enhanced motor activity subsided after 20 to 30 min, and all mice then resumed normal behavior. Each compound was quite effective in reducing liver, kidney, intestine, and spleen Cd levels. The major differences noted were in lung and...
testes. In contrast to the actions of DeDTC and DmDTC, DiDTC did not promote accumulation of Cd in lung, and it reduced significantly the testicular Cd burden. In addition, none of the gross pathological changes observed upon necropsy of the acutely poisoned mice was observed with the chronically Cd-loaded mice. In view of the fact that whole-body gamma counting of mice, performed one day prior to sacrifice, revealed that DiDTC was only minimally effective in reducing total body burden (figure 2), the significant decreases in liver and kidney Cd burdens were unexpected.

One day after the 13th injection of each compound, whole-body gamma counting was performed as before (figure 2); one day later, selected organs were removed for determination of radioactivity. However, because results of whole-body gamma counting suggested that Cd was being redistributed to other tissue and was not being excreted as rapidly following DiDTC treatment, samples of femur, skin (with hair), and striated muscle (thigh) were also removed for determination of Cd content.

It can be seen from figure 3 that after 13 injections, liver, kidney, and spleen Cd burdens had been further reduced, and there were no remarkable differences in the enhanced brain Cd uptake which was observed in the earlier studies.5,6

The influence of each chelator on the Cd content of bone, skin, and muscle is shown in figure 4, expressed as percent of administered Cd per gram of tissue. Bone Cd content was significantly reduced by DeDTC and DmDTC, but not by DiDTC. Skin concentration was not altered by any of the three compounds. However, all analogs markedly enhanced the muscle Cd concentrations, and the greatest increase was noted following DiDTC treatment. In view of the fact that

![Figure 2](image-url)

**Figure 2.** Whole-body retention of Cd in mice which received seven (A) and 13 (B) injections of DeDTC, DmDTC, and DiDTC, each at 2.22 mmole per kg per injection. Data are expressed as mean (n = 12) ± 1.0 S.D. Levels of statistical differences were determined by ANOVA in comparison with each appropriate control group.
FIGURE 3. Same as figure 1, except mice received a total of 13 injections of each compound, 2.22 mmoles per kg per injection. Data are expressed as mean (n = 6) + or − 1.0 S.D.

FIGURE 4. Effects of 13 injections of DeDTC, DmDTC, and DiDTC, each at 2.22 mmoles per kg per injection, on Cd levels in bone (Bo), skin with hair (Sk), and striated muscle (Mu). Data are expressed as mean (n = 6) + or − 1.0 S.D. Levels of statistical differences were determined by ANOVA in comparison with each appropriate control group.

the striated muscle mass constitutes a significant proportion of the total body weight, it probably explains the relatively lower efficacy of DiDTC in reducing total body Cd burden (figure 2).

Results of excretion studies (figure 5) were altogether compatible with data obtained from the whole-body counts (figure 2). Control mice excreted slightly more than 0.1 percent per day. Daily treatment with DiDTC for three days resulted in an excretion rate of about 0.55 percent per day, while treatment with DeDTC or DmDTC enhanced the rate to 1.3 to 1.5 percent per day. As observed earlier,5,6 excretion was exclusively by the fecal route.

Discussion

In spite of only subtle quantitative differences in the reactivity of each of the dithiocarbamates with various cations when assessed in aqueous solution in vitro, it is apparent that there are some rather striking differences in vivo. This first became evident in the antidotal studies of acute Cd poisoning, in which it was apparent that DiDTC seemed to
enhance Cd toxicity when given prior to Cd, and was only marginally protective when treatment was delayed for two hrs after Cd administration. Even though 25 percent of the mice survived the latter treatment regimen, the massive gross pathology observed in the surviving animals certainly precludes this compound as a potential drug for acute Cd poisoning. In contrast, DmDTC was fully antidotal when administered 30 min before and up to two hrs after the >LD<sub>100</sub> dose of Cd, and the organ changes observed were generally mild except for those mice which had received the chelator 30 min prior to Cd. In confirmation of previous work, DeDTC was not a totally effective antidote when used prophylactically, but was effective when treatment was delayed 30 min to two hrs after Cd administration. In addition, the degree of gross pathology observed at necropsy was detectably less following DeDTC treatment than was noted following treatment with DmDTC or DiDTC.

In regard to promotion of mobilization and excretion of metallothionein-bound Cd, DeDTC and DmDTC were remarkably similar, both qualitatively and quantitatively. The actions of DiDTC on hepatic, renal, intestinal, and splenic Cd burdens were very similar to those of DeDTC and DmDTC. However, there were five quite marked differences noted following treatment with DiDTC: (1) it did not enhance redistribution of Cd to lung; (2) it reduced rather than enhanced the testicular Cd burden; (3) it promoted a much greater redistribution of Cd to striated muscle; (4) it did not reduce the bone Cd burden; and (5) it increased the rate of fecal excretion of Cd much less than did the other two analogs. In addition, the notable absence of gross pathology upon dissection of DiDTC-treated mice in this series of experiments was in contrast to the severe pathology observed upon necropsy of the surviving animals in the acute toxicity studies.

All three drugs promoted a marked redistribution of Cd to the brains of treated animals, an action observed previously following DeDTC treatment of Cd-laden mice. As the bioavailability of Cd from the complex in the brain is not known, the toxicological significance of this accumulation cannot be assessed on the basis of the present data. However, as Cd ion administered s.c. is much more toxic to brains of neonatal mice than to mature mice, presumably owing to incomplete development of the blood-brain barrier.
in the neonates,15 the tendency of the dithiocarbamates to promote this accumulation may militate against their potential clinical application in treating acute or chronic Cd intoxication.

It was reported that the Cd-DeDTC complex, (DeDTC)₂Cd, was not detectably soluble in water, serum, dilute acid or alkali, or carbon tetrachloride;7 therefore, the enhanced brain Cd levels of DeDTC-treated animals seemed to pose an enigma. However, Cantilen et al2 recently reported on the pharmacodynamics of Cd redistribution to brain. They considered and ruled out experimentally the possibility that DeDTC may cause a generalized increase in cerebral capillary permeability to a diffusion-limited substance, or that it may reduce rate of cerebral blood flow thereby prolonging cerebral circulatory transit time. They next considered the possibility that DeDTC may chelate Cd to form a complex which would more readily cross the blood-brain barrier. Using the classical octanol/water partition coefficient method, they showed that the Cd ion alone had an octanol/water partition coefficient of 0.03 ± 0.002. When a four-fold molar excess of DeDTC was added to the buffered aqueous Cd solution prior to addition of octanol, the coefficient found after partitioning was 11.34 ± 0.5, or greater than a 375-fold increase.2 It thus appears that as the dithiocarbamates are extremely active in mobilizing Cd from the liver and kidney, there is a need to synthesize and evaluate selected analogs which, when complexed with Cd, would still contain one or more polar or lipophobic groups which could be expected to suppress the capability of the complex to traverse the blood-brain barrier.

Finally, a caveat may be in order for anyone involved in studies of equimolar doses of analogs, irrespective of the nature of the study: one must be reasonably certain that any compound is indeed substantially what it is alleged to be. As mentioned in the Materials and Methods section, the dimethyldithiocarbamic acid sodium salt as received bore the label of a highly reputable manufacturer, was well sealed, and was imprinted with the structural formula and molecular weight. It of course was also clearly labeled as being not for drug use. There was no indication of water content. Water analysis by a commercial laboratory showed that the material contained over 40 percent water. If this compound had been used in these studies without considering the possibility of a significant water content, the doses administered would have been only about 60 percent of those intended, and spurious data could have been obtained.

References


