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Structure-Activity Relationships Among Dithiocarbamate Antidotes for Acute Cadmium Chloride Intoxication

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Eight sodium dithiocarbamates ($NaS_2CNR_1R_2$) have been examined as antidotes for acute cadmium intoxication. While all of them possess an ability to increase survival when given to mice 2 hr after a lethal (>99%) intraperitoneal injection of 10 mg/kg of $CdCl_2 \cdot 2.5H_2O$, their effects on the organ distribution of cadmium vary considerably. It has been possible to show that the accumulation of cadmium in the brain and kidney as well as the survival rates can be correlated with a numerical measure of the polarity of the groups R_1 and R_2. Each factor has a different dependence on the polarity, but it is possible to construct a composite factor for antidotal efficacy which incorporates survival rate, brain cadmium levels and kidney cadmium levels. The factor constructed here exhibits an optimal value approximately in the middle of the polarity range studied. Compounds which have $R_1 = -CH_2CH_1OH$ and $R_2 = -CH_2CH_2OH$, or $-CH_3$ or $-C_2H_5$ appear to be the most effective antidotes of the compounds examined.

Introduction

The discovery in 1981 by Gale and his coworkers (*1*) that sodium diethyldithiocarbamate was an effective antidote for acute cadmium intoxication, even when its administration was delayed until as much as 5 hr after the administration of a lethal (>99%) dose of cadmium chloride, represented a new departure in the search for chelate antidotes for cadmium intoxication. This finding has been confirmed and extended to a number of structurally related dithiocarbamates (*2–4*) in the search for compounds providing a greater degree of control over the redistribution of cadmium subsequent to the administration of the antidote. One major problem encountered with the use of sodium diethyldithiocarbamate as an antidote for cadmium intoxication is its tendency to increase the cadmium content of the brain (*2,3,5*). This is presumably related to the ability of diethyldithiocarbamate to form lipid-soluble complexes with cadmium (*5*). If this could be controlled by structural modifications, the resulting compounds should be more satisfactory antidotes for cadmium intoxication. We have attempted to put this and other aspects of the behavior of these antidotes on a quantitative basis. For this purpose we have carried out a structure-activity analysis on these compounds, using structural parameters provided by the studies of Hansch and Leo (*6*) to characterize the relative polarity of the various dithiocarbamates studied. In the current work we have used only the sodium salts of these compounds, rather than the more readily crystallized ammonium salts used in our earlier study (*3*). This avoids the problems which arise from the limited ability of the cadmium-damaged kidney to handle ammonium salts. The goal of the current study was to search for relationships which existed between the structural parameters characteristic of the compounds and various measures of the antidotal activity which they exhibited. It was believed that this would allow a more rational selection of one or two of the compounds for more detailed examination and furnish a guide in the selection of new compounds for synthesis.

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Methods

The methods used in the animal studies were patterned after those of Gale et al. (*1*), except that the animals were sacrificed 2 weeks rather than 4 weeks after the administration of the cadmium.

Male, ICR mice, weighing 30 ± 4 g, obtained from Harlan Industries, Indianapolis, Indiana, were acclimated for 1 week before experimental use. They were allowed food and water *ad libitum*. A lethal (>99%) dose of $CdCl_2 \cdot 2.5H_2O$ was given IP at a level of 10 mg/kg. Two hours later, each animal was given a single IP injection of the dithiocarbamate at a level of 2.2 mmole/kg, with an injectate volume of 0.1 or 0.2 mL, depending on solubility. The pH was controlled for antidotes with a high pH by adding a small chunk of dry ice to the solution to bring the pH down close to 7. Sodium iminodiacetic acid dithiocarbamate was given as a 1:1 Ca^{2+} salt to prevent tetany. After 2 weeks of observation the animals were sacrificed by cervical dislocation and the cadmium content determined on samples of brain, kidney and liver, which had been digested in nitric acid, using flame atomic absorption spectrophotometry.

The dithiocarbamates used in this study were prepared by using literature methods (*9,10*) and were analyzed to contain 98.5% or better of the indicated sodium dithiocarbamate. Only sodium salts were used as these were found to produce fewer complications and a reduced mortality in comparison with the more readily crystallizable ammonium salts.

Results

The results obtained with the eight compounds selected are presented in Table 1, which also gives the structure of each of the compounds used. The mice administered the dithiocarbamate antidotes which were less effective in removing cadmium from the animal, sodium sarcosine dithiocarbamate (IV), sodium iminodiacetic acid dithiocarbamate (VI), sodium ethyl(hydroxyethyl)dithiocarbamate (VII) and sodium propyl(hydroxyethyl)dithiocarbamate (VIII) showed a tendency to have darkened, swollen livers, one kidney embedded in the liver, and adhesions of liver or intestine to the peritoneum. The better antidotes, sodium diethyldithiocarbamate (I), sodium dimethyldithiocarbamate (II), sodium methyl(hydroxyethyl)dithiocarbamate (V) and sodium di(hydroxyethyl)dithiocarbamate (III) produced fewer of these gross abnormalities.

Cadmium was found to be present in the brain of mice given sodium diethyldithiocarbamate (I) and sodium dimethyldithiocarbamate (II) at levels of 2.6 and 2.3 ppm, respectively.

The effect of increased time intervals between the administration of sodium di(hydroxyethyl) dithiocarbamate (I) and that of the cadmium chloride is shown in Table 2.

Discussion

The data presented in Table 1 show that the differences in the behavior of these dithiocarbamates is considerable. The variation in the amounts of cadmium remaining in the kidney and the brain is quite striking. The data on the

Table 1. Results of structure-activity studies.

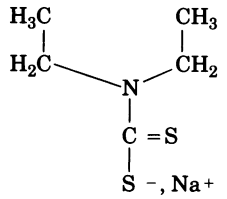
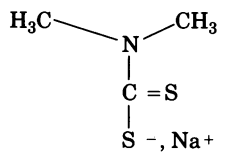
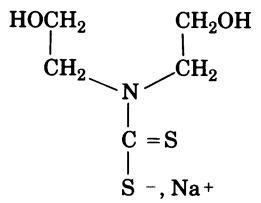
	Compound	Dose, mmole/kg	Survival	Liver Cd, ppm	Kidney Cd, ppm	Brain Cd, ppm
I		2.2	8/8	27.7 ± 6.0	20.6 ± 6.5	2.6 ± 1.6
II		2.2	8/8	16.9 ± 3.0	20.4 ± 9.5	2.3 ± 0.5
III		2.2	8/8	18.1 ± 7.4	14.2 ± 5.7	0

Table 1. Results of structure-activity studies (Continued).

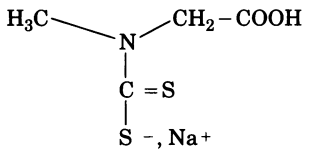
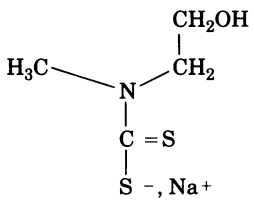
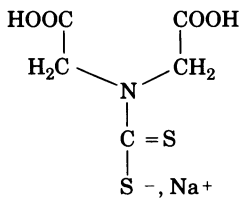
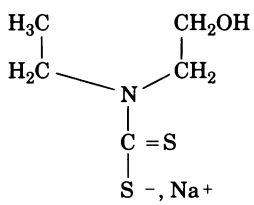
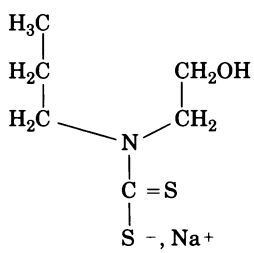
	Compound	Dose, mmole/kg	Survival	Liver Cd, ppm	Kidney Cd, ppm	Brain Cd, ppm
IV		2.2	8/8	26.6 ± 9.2	29.0 ± 12.4	0
V		2.2	8/8	26.6 ± 4.7	18.3 ± 5.9	0
VI		2.2	7/8	25.3 ± 7.8	25.0 ± 12.6	0
VII		2.2	8/8	24.4 ± 2.2	13.9 ± 5.8	0
VIII		2.2	8/8	25.1 ± 2.1	26.8 ± 5.1	0

Table 2. Effect of time interval on efficiency of sodium di(hydroxyethyl) dithiocarbamate.[a]

$CdCl_2 \cdot 2.5\ H_2O$ (IP), mg/kg	Time interval after cadmium, min	Antidote dosage, mmole/kg	Survival rate[b]	Liver Cd, ppm	Kidney Cd, ppm	Brain Cd, ppm
10	20 min	2.2	8/8	12.0 ± 7.5	6.9 ± 6.2	–0–
10	120	2.2	8/8	18.1 ± 7.4	14.2 ± 5.7	–0–
10	480	2.2	4/8	30.8 ± 6.7	10.5 ± 6.7	–0–

[a]Organ cadmium contents were determined at the end of 2 weeks.
[b]All cadmium-only control mice died within the first week.

diisopropyl derivative, available from the study of Gale and his co-workers (*4*), allow an extrapolation of our data to compounds in which the substituents on the nitrogen atom are longer organic chains. The data in Table 1 suggest that the changes in the relative hydrophobic/hydrophilic character of the groups attached to the nitrogen atom may be important in governing the trends observed. In order to obtain a quantitative estimate of the relative hydrophobicity of the R groups in each compound, the sum of the π factors of the R groups, as determined by Hansch and Leo (*6*), was used. These π factors were developed to allow the estimation of the octanol/water partition coefficients for organic compounds on the basis of numerical parameters assigned to the various structural constituents. In the present case, the only difference between the compounds lies in their R groups, so the sum of the π factors of the R groups alone was used to estimate the relative differences in hydrophobicity of the dithiocarbamates used (Table 3).

Figure 1 shows a plot of the survival ratio versus $\Sigma\pi$. It is quite interesting that all of these dithiocarbamates show some antidotal activity under the conditions used. There is a decrease in the antidotal action for the most hydrophobic of the compounds, the diisopropyl derivative. The fact that some of these compounds facilitate the entry of cadmium into the brain is an obvious disadvantage. The relative amount of cadmium in the brain is plotted against $\Sigma\pi$ in Figure 2. This amount appears to increase when $\Sigma\pi$ increases above about +0.25. The relative amount of cadmium remaining in the kidney is also a parameter that presumably should be low. The dependence of this on $\Sigma\pi$ is shown in Figure 3.

The net efficacy of these compounds should be represented by a parameter which combines the favorable and unfavorable aspects of the behavior of these compounds. The construction of such a parameter is somewhat arbitrary but, in general, it should increase as the desirable actions are more prominent and decrease as the adverse effects become more evident. The combination which we selected to represent this overall antidotal efficacy of these compounds is:

$$\text{Efficacy} = \text{SR} - (\text{Rel. Br Cd}/2) - (\text{Rel. Kid. Cd}/2)$$

where SR is survival ratio, Rel. Br. Cd/2 is relative brain cadmium ÷ 2 and Rel. Kid. Cd/2 = relative kidney cadmium ÷ 2.

This overall efficacy is plotted against $\Sigma\pi$ in

Table 3. π parameters for dithiocarbamates studied.[a]

Compound	R_1	R_2	$\Sigma\pi$
I	C_2H_5	C_2H_5	2.04
II	CH_3	CH_3	1.12
III	C_2H_4OH	C_2H_4OH	− 1.54
IV	CH_3	CH_2COOH	− 2.44
V	CH_3	C_2H_4OH	− 0.21
VI	CH_2COOH	CH_2COOH	− 3.72
VII	C_2H_5	C_2H_4OH	+ 0.25
VIII	C_3H_8	C_2H_4OH	+ 0.78

[a]The values for the substituent constants of the individual groups are those given by Hansch and Leo (*6*). $\Sigma\pi$ for a compound is simply the sum of the π values given for each of the R groups; thus for compound V, $\Sigma\pi = \pi(CH_3) + \pi(CH_2CH_2OH) = 0.56 + -0.77 = 0.21$. The introduction of a negative charge leads to a contribution of −2.28 to $\Sigma\pi$.

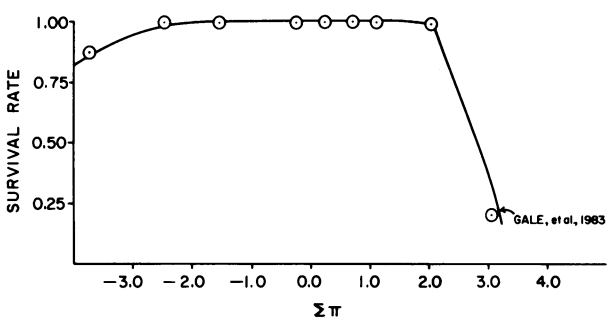


FIGURE 1. Survival rates vs. $\Sigma\pi$. The data labeled Gale et al., 1983, in this figure and in Figures 2 and 3 are from ref. (*4*).

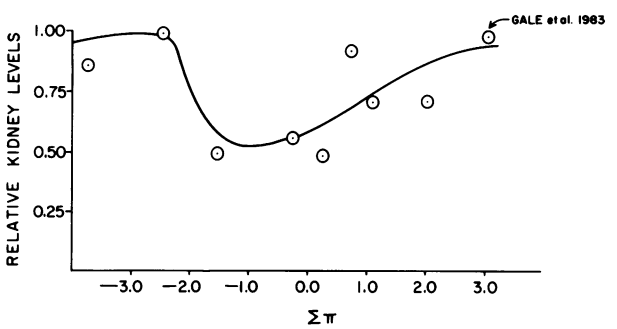


FIGURE 2. Relative kidney levels vs. $\Sigma\pi$.

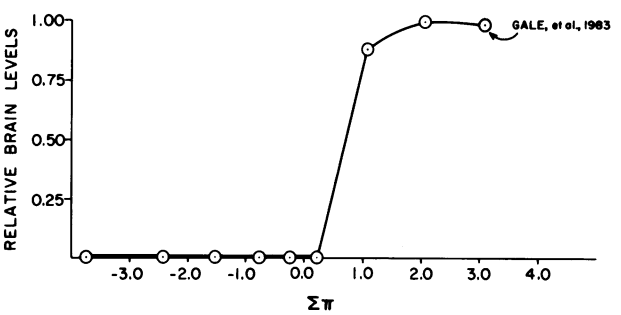


FIGURE 3. Relative brain levels vs. $\Sigma\pi$.

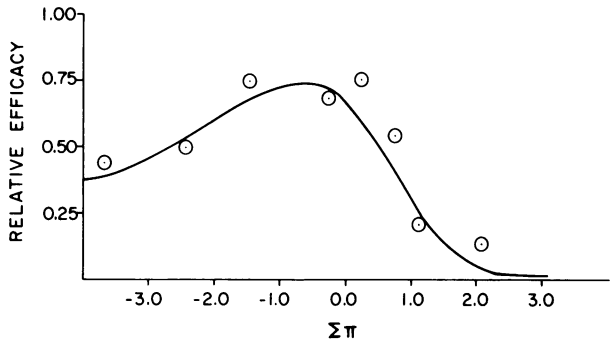


FIGURE 4. Relative efficacy vs. $\Sigma\pi$. The relative efficacy used here as the ordinate is defined as: survival rate − (relative kidney cadmium)/2 − (relative brain cadmium)/2.

Figure 4. While there is some scatter in the plot, it hints strongly that compounds in the central portion of the $\Sigma\pi$ range possess that combination of properties which provide for an optimal efficacy, using the definition of efficacy given above.

The fact that there was a 2-hr interval between the administration of cadmium and that of the antidote is also of possible special significance. A compound effective under such conditions is apparently able to remove cadmium from binding sites from which it cannot be easily removed by EDTA or DTPA. Numerous studies have shown that EDTA and DTPA lose at least part of their antidotal efficacy unless given almost immediately after the cadmium (*9–12*). It has also been demonstrated that this falloff in antidotal efficacy occurs at a more rapid rate than that at which metallothionein synthesis is induced in the liver (*13*). The dithiocarbamates used in the present study may be able to remove cadmium from those sites which are responsible for the falloff in the antidotal efficacy of EDTA type chelators. These sites may well be intracellular ones to which EDTA or DTPA do not have access (*13*).

The analysis of antidotal efficacy presented here is quite different from the usual one which is based solely on the relative values of the stability constants of the various metal complexes involved (*14*). The apparent reason for this is the very high probability that these compounds can chelate to cadmium which is intracellular in nature, while the typical chelating agent of the EDTA type or other highly ionized sort has an extremely limited ability to penetrate the cell membrane. It would appear possible then that the factors governing the relative efficacy of chelating agents which are to act intracellularly are significantly more complex than those governing chelating agents whose action is solely extracellular.

Sodium diethyldithiocarbamate—and presumably its less thoroughly studied analogs used here—differs in some important ways from the majority of the chelating agents which are used therapeutically. The dithiocarbamates are metabolized quite rapidly to a variety of products including the disulfuram derivatives, carbon disulfide and the parent amine among others (*16,17*). The ability of this compound to gain entry into cells can be inferred from its effectiveness as an antidote for tetracarbonylnickel(O), $Ni(CO)_4$ (*18*). This latter compound has a low boiling point and behaves much like a nonpolar organic compound when administered to rats (*19*). The fact (*18*) that sodium diethyldithiocarbamate is an effective antagonist to nickel carbonyl when given several hours after exposure to $Ni(CO_4)$ indicates that its distribution in the human body mimics, at least in part, that of $Ni(CO)_4$. Its behavior in the case of cadmium-intoxicated animals is also consistent with an ability to get into cells in which part of the cadmium has been taken up. Because sodium diethyldithiocarbamate is charged, it may be that this transport through the cell membrane is accomplished by some of its metabolic products which then revert to the dithiocarbamate.

The data presented in Table 1 and the mode of action proposed for these compounds suggest that they might be effective even when the delay between administration of the cadmium and the antidote is significantly greater than 2 hr. Gale and his co-workers (*1*) have presented evidence showing that diethyldithiocarbamate can have a very pronounced antidotal action even when its administration is delayed as much as 6 hours. We carried out analogous experiments on sodium di(hydroxyethyl)dithiocarbamate, and these results, shown in Table 2, indicate that this compound also exhibits considerable antidotal activity when its administration is delayed by as much as 8 hr. When animals are given 10 mg $CdCl_2 \cdot 2.5H_2O$ intraperitoneally, and no antidote, none survive to the end of the 2-week period used in these experiments.

The analysis presented here has been used to select compounds for testing in chronic cadmium intoxication in mice. Results obtained to date (*15*) confirm the ability of dithiocarbamates such as sodium di(hydroxyethyl)dithiocarbamate to mobilize cadmium, even from aged deposits, without increasing the concentration of cadmium in the brain. Should these compounds prove to have no untoward long-term effects, they may provide a vehicle for the mobilization of cadmium in chronic human cadmium intoxication. The use of parameters related to the partition coefficient of

the chelating agent and its metal complexes as developed here may also prove useful in other cases where chelating agents are needed to mobilize metals from intracellular sites. This type of situation is found in patients with chronic metal poisoning due to long-term environmental exposure as well as those with iron-overload due to a long series of blood transfusions (*20*), hepatolenticular degeneration with its accumulation of copper (*21*) or in thallium intoxication, where injested thallium replaces the potassium ion (*22*).

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