Effects of Combined Treatment with Diethyldithiocarbamate and Diethylenetriaminepentaacetate on Organ Distribution and Excretion of Cadmium*

GLEN R. GALE, PH.D.,†‡ LORETTA M. ATKINS, B.S.,§ ERNEST M. WALKER, JR., M.D., PH.D.,§ and ALAYNE B. SMITH, B.S.†

†Veterans Administration Medical Center, ‡Department of Pharmacology, and §Department of Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29403

ABSTRACT

Diethyldithiocarbamate (DDTC) and diethylenetriaminepentaacetate (DTPA) were assessed to determine if combination treatment with these two chelators of different chemical classes would enhance mobilization and excretion of metallothionein-bound cadmium (Cd) from selected organs of mice which had earlier received 0.03 mg of CdCl₂·2.5 H₂O along with 1.0 μCi of ¹⁰⁹Cd. In addition to measuring individual organ radioactivity after seven and after 13 injections of each compound individually as well as in combination, whole body Cd burden was measured, and the routes and rates of Cd excretion were determined. When used alone, DDTC was effective in mobilizing Cd from kidney, liver, intestine, and spleen. The DTPA when used alone was not consistently effective in reducing Cd burdens in any of the organs assessed. Coadministration of DDTC and DTPA promoted an enhancement of Cd mobilization from liver, kidney, spleen, and intestine over that which was observed with DDTC alone. When DTPA was administered with DDTC, it did not prevent accumulation of Cd in lung and brain which was observed upon treatment with DDTC alone. Combined treatment did produce a more marked depletion of total body ¹⁰⁹Cd burden than did the administration of DDTC alone. A more rapid rate of both fecal and urinary excretion of Cd was observed when the chelators were coadministered. It was concluded that at least an additive or possibly supraadditive effect may be obtained by combining a dithiocarbamate chelator with one of the aminocarboxylate class in total body Cd decorporation.

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Introduction

Numerous chelators have been tested as antagonists of acute cadmium (Cd) toxicity as well as for their efficacies in mobilizing metallothionein-bound Cd; however, the different animal species used and the diverse schedules of chelator administration in relation to the time of Cd intoxication have sometimes made close comparisons difficult. In spite of these differences, it is evident that dimercaptosuccinate (DMSA), diethylenetriaminepentacetate (DTPA), and diethyldithiocarbamate (DDTC) are all effective under specific experimental conditions.

When given to mice within 20 min after a lethal dose of Cd, DMSA reduces mortality markedly. It also reduces Cd levels in liver, kidneys, testes, and brain, and promotes urinary excretion. However, when mice are given a non-lethal dose of Cd along with $^{109}$Cd, then held for a period of several weeks to attain a virtually steady state of Cd sequestration with metallothionein, DMSA is totally ineffective in reducing total body burden or Cd content of any of seven organs assessed.

The aminocarboxylate chelator, DTPA, was found in two studies to be highly effective in enhancing survival in mice acutely poisoned with Cd. When given immediately after an LD$^{100}$ dose of Cd, it was even more effective than DMSA in reducing Cd burdens of liver, kidneys, testes, and brain, as well as increasing the rate of urinary Cd excretion. In another study, rats were injected i.p. with a low dose of $^{109}$Cd daily for four days, then three days later were given DTPA, alone or in combination with 2,3-dimercaprol, five days a week for two weeks. The DTPA alone did not reduce significantly the Cd content of any of eight organs assessed. When given in combination with dimercaprol, it potentiated slightly the action of the latter in reducing renal Cd burden. Other investigators showed that DTPA is moderately effective in mobilizing metallothionein-bound Cd from kidney and in promoting its urinary and fecal excretion, but does not reduce the Cd content of any of six other organs evaluated.

In two studies in which DDTC was given to mice immediately after or 20 min after a lethal dose of Cd, it was reported to be ineffective or moderately effective in increasing survival. However, in yet another study, DDTC was shown to be fully protective in mice which received a $>LD^{100}$ dose of Cd, but only if DDTC treatment was delayed for 30 min to about five hrs after Cd administration. This phenomenon of enhanced antidotal effectiveness with delayed treatment has been confirmed by other investigators and contrasts strikingly with the decreased effectiveness of DMSA, DTPA, and ethylenediaminetetraacetate with time after acute Cd poisoning. In addition, DDTC rapidly promotes excretion of metallothionein-bound Cd from liver, kidney, spleen, and intestine; however, it concomitantly increases moderately the levels of Cd in lung, testes, and heart, and increases the brain Cd levels over ten-fold. On an equimolar dose basis, it is much more effective than DTPA in reducing the renal Cd burden. Mobilization of metallothionein-bound Cd from liver, kidney, and spleen appeared to proceed via first order kinetics; i.e., a relatively constant percentage of the administered dose was mobilized rather than a constant amount. Redistribution of Cd to brains of DDTC-treated mice is presumably a consequence of the high octanol/water partition coefficient of the Cd-DDTC complex; the reported numerical quotients correspond to log$_{10}$P values of -1.52 for Cd ion and 1.05 for the Cd-DDTC complex. Even though Cd-bearing mice treated with DDTC displayed no detectable behavioral aberrations, the toxicological significance of the
enhanced brain Cd burden has not yet been explored.

The study reported here was initiated to determine if coadministration of DTPA with DDTC would increase the rate of mobilization and excretion of metallothionein-bound Cd, and if such coadministration would reduce the extent of redistribution of Cd to brains of mice.

Materials and Methods

The strain of mice used, the procedures for administration of Cd and chelators, methods of $^{109}$Cd measurements, and statistical methods have been described in detail.$^6,^7,^8$ Briefly, male BDF$_1$ mice* were given an i.p. injection of 0.03 mg CdCl$_2$·2.5 H$_2$O† in 1.0 ml of 0.9 percent NaCl solution which also contained 1.0 µCi of $^{109}$CdCl$_2$‡ per ml. After 14 days, unless indicated otherwise, the mice to be used for distribution studies and whole body gamma counting were started on a regimen of DDTC† alone, DTPA§ alone, or DDTC followed 15 min later by DTPA. The DDTC was dissolved in 1.0 percent NaHCO$_3$. The DTPA solution was prepared as the ZnNa$_3$ salt in view of the lesser toxicity of DTPA when given as the ZnNa$_3$ salt rather than as the CaNa$_3$ salt.$^{14,15}$ Each chelator, either used alone or coadministered, was given at a dose of 2.0 mmole per kg per injection. Injections were on a thrice weekly regimen, and mice were subjected to whole body gamma counting after seven and 13 injections. Similarly, after the same number of injections, six mice from each group were sacrificed by cervical dislocation, and selected organs were removed for measurement of radioactivity using a gamma well counter.

For excretion studies, 14 days after administration of Cd with $^{109}$Cd, mice were divided into four groups of six each. Six served as controls, six were given DDTC, six were given DTPA, and six were given DDTC with DTPA on days 0, 1, and 2. Each compound was given at a dose of 2.0 mmole per kg. Mice were housed in plastic metabolism cages.$^7$ Total feces and urine were collected on days 1, 2, and 3. After measuring radioactivity, data were expressed as percent of the administered Cd excreted per mouse per day.

Results and Discussion

Distribution and Mobilization Studies

The effects of seven injections of DDTC and DTPA, individually and coadministered, on Cd content of eight selected mouse organs are shown in figure 1. The responses to DDTC were qualitatively the same as observed earlier,$^6,^7,^8,^9$ with only subtle quantitative differences. In contrast, DTPA caused statistically significant but relatively modest decreases in renal, intestinal, testicular, and myocardial Cd burdens. It neither promoted Cd mobilization from liver, nor did it redistribute the metal so as to cause elevated levels in lung and in brain. When DDTC was coadministered with DTPA, there was a somewhat greater extent of mobilization than was obtained with DDTC alone. Analysis of variance (ANOVA) of the DDTC groups with the DDTC plus DTPA groups showed that these increases were statistically significant in the liver ($p = 0.007$), kidney ($p = 0.001$), spleen ($p = 0.05$), and intestine ($p = 0.013$). Combined treatment did not prevent the redistribution which resulted in increased Cd levels in lung, heart, and brain.

The results obtained following 13 injections of each chelator are shown in figure 2. Only slight quantitative differences can be discerned in comparison
Figure 1. Effects of seven injections of DDTC, DTPA, and DDTC plus DTPA on Cd levels in selected mouse organs expressed as percent of Cd administered. Treatment with each compound was on a thrice weekly schedule, and each was given at a dose of 2.0 mmole per kg. Data are expressed as mean (n = 6) + 1.0 S.D. Organs assessed were liver (Li), kidneys (Ki), intestine (In), spleen (Sp), lungs (Lu), testes (Te), heart (He), and brain (Br). Levels of statistical differences were determined by analysis of variance (ANOVA) in comparison with each appropriate control group.

Figure 2. Same as for figure 1, except mice received a total of 13 injections.

with figure 1, and ANOVA showed that the two compounds when coadministered continued to cause a significantly greater mobilization from liver (p = 0.013), kidney (p = <0.001), and intestine (p = 0.001) than was obtained with DDTC alone.

In our prior studies, the standardized model has been used in which mice were treated for a total of 13 injections on a thrice weekly schedule beginning at least 14 days after administration of 0.03 mg of CdCl₂·2.5 H₂O with ¹⁰⁶Cd. No study had been made of the organ Cd levels at any time prior to seven injections of DDTC or certain of its analogs. As the greatest reduction of Cd levels has consistently been in kidney, this organ was assessed for Cd content following only one injection and again after three daily injections of each compound, individually and in combination. Results are shown in figure 3. A rather impressive 44 percent of the renal Cd
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Figure 3. Effects of one injection and of three injections of DDTC, DTPA, and DDTC plus DTPA on renal Cd levels. Doses and statistical procedures were as described for figure 1.

was removed by only one injection of DDTC at 2.0 mmole per kg, and this was increased to 68 percent after three daily injections; however, this effect was not influenced by coadministration of DTPA.

Excretion Studies

The results of whole body gamma counting of mice following seven and 13 injections, expressed as percent of administered Cd, are shown in figure 4. After seven injections, the total body Cd load was significantly lower than controls with each of the treatment regimens. The greater reduction obtained following coadministration of DTPA with DDTC (17 percent) was approximately equal to the sum of the reductions when each was given individually (DDTC, 9 percent; DTPA, 5 percent), and the difference between the DDTC plus DTPA group, when compared by ANOVA with data from each of the other treated groups, was highly significant ($p < 0.001$). Following 13 injections, individually and in combination, the reductions were even greater with one exception: the DDTC alone effected a 26 percent reduction, but the reduction conferred by DTPA was not significantly different from the mean control value. Coadministration yielded a 38 percent reduction in total body Cd burden which was significantly greater ($p < 0.001$) than obtained with DDTC alone.

The routes and rates of cumulative Cd excretion obtained with each treatment regimen are shown in figure 5. Only trace levels of $^{109}$Cd were measurable in urine from control mice or from mice treated with either chelator alone. However, coadministration yielded an initially rapid rate of urinary excretion, which appeared to approach the control rate over the three-day period of measurements. In control mice, as well as in those treated
with DTPA alone, there was a slight but measurable amount of $^{109}$Cd excreted in feces which in control mice proceeded at about the same rate as reported earlier.\(^6\)

Fecal excretion was markedly enhanced by DDTC. Concomitantly with a rapid initial rate of urinary excretion elicited by DDTC plus DTPA which decreased over the period of measurements there was a gradual increase in rate of fecal excretion. These patterns of excretion suggest that metallothionein-bound Cd is multicompartmented, and one of these compartments, even though it comprises only a relatively small percentage of the total cadmium burden, is quite responsive to combined treatment with these two compounds.

Even though these data do not reveal that any striking synergistic action can be obtained by combining an aminocarboxylate chelator with one of the dithiocarbamic acid class, they do indicate that at least an additive or possibly supradditive effect (figure 4) can be obtained in total body Cd decorporation.

References


