Bull. Environm. Contam. Toxicol. 22,617-624 (1979)

Lead Decorporation Following Therapy with the Dibutyl Ester of Diethylenetriaminepentaacetate in the Mouse

Elsie M. B. Sorensen[1], Elizabeth S. Moretti, and Arthur Lindenbaum
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill. 60439

Once lead enters the brain of young mammals, it apparently becomes tightly bound and remains sequestered behind the blood-brain barrier for a long time (GOLDSTEIN and DIAMOND 1974). Low-level exposures to lead (as the nitrate or acetate) result in higher concentrations of lead in the brain of the younger rat or primate than in the brain of the older animal (MOMČILOVIĆ and KOSTIAL 1974). Although marked decreases in lead levels in the blood and other tissues follow cessation of exposure, lead deposition in the brain is refractory (HAMMOND et al. 1967). Even with chelation therapy there is either no decline in brain lead concentrations (HAMMOND 1971, 1973; GOLDSTEIN and DIAMOND 1974) or only small decreases (CASTELLINO and ALOJ 1965; HAMMOND et al. 1967; HAMMOND 1971; HOFMANN and SEGEWITZ 1975). Lead exposure in children or rats can lead to neuronal damage even before symptoms are evident (DE LA BURDE and CHOATE 1972; MICHAELSON and SAUERHOFF 1974).

Currently, clinical treatment of symptomatic lead poisoning consists primarily of administration of the chelating agent calcium disodium ethylenediaminetetraacetate (EDTA) (BRUGSCH et al. 1965; STRICKLAND et al. 1976). Unfortunately, because EDTA is highly hydrophilic, it remains almost exclusively extracellular (CASTELLINO and ALOJ 1965) and removes little or no lead from the brain. Although the structurally similar diethylenetriaminepentaacetate (DTPA) molecule is also limited primarily to extracellular spaces, it is more effective than EDTA in removing lead from the rat (HAMMOND 1971) because of a higher binding affinity for lead (SILLÉN and MARTELL 1964) and a somewhat slower clearance from the body (FOREMAN 1960).

In previous work to increase the effectiveness of unesterified DTPA for removal of actinides, the dibutyl ester of DTPA (Bu_2DTPA) was synthesized (GUILMETTE et al. in press). Because this molecule has a reduced charge and increased lipid solubility compared to either EDTA or DTPA, it should be more capable of crossing the blood-brain barrier, be potentially more effective in reducing otherwise refractory brain burdens of lead, and possibly more effective in reversing brain damage. We therefore

[1]Present address: Department of Biology, Memphis State University, Memphis, Tennessee 38152.

0007-4861/79/0022-0617 $01.60

tested the therapeutic efficacy of this ester, using both mature and immature mice with a well-established lead burden, the latter to simulate treatment of young children following lead poisoning. This study was designed to determine whether the dibutyl ester can mobilize more lead from mouse tissues compared to DTPA and EDTA, whether it promotes urinary or fecal excretion of lead, and whether any prolonged effect occurs after discontinuation of treatment.

MATERIALS AND METHODS

To compare the efficacy of Bu_2DTPA, DTPA, and EDTA, five groups of four to eight female BCF_1/Anl mice (149 to 177 days of age and 20 to 22 g in weight) received a lateral tail vein injection of lead citrate (30 mg Pb/kg, 81 μCi ^{210}Pb/kg) in a 3.75 mg Pb/mL solution. At 4 h, one groups was sacrificed and tissues removed for determination of lead at initiation of treatment; three other groups of mice received the first of three intraperitoneal injections of the calcium chelates of either Bu_2DTPA, DTPA, or EDTA (0.25 mmoles/kg). One group of mice was administered an equivalent volume of 0.9% saline intraperitoneally. Therapy or saline injections were repeated two additional times at 18 and 24 h. Control and treated mice were sacrificed at 48 h following lead injection. Tissues were removed and analyzed for lead as measured by the 0.047 MeV gamma emission from ^{210}Pb on a Beckman 310 Gamma Radiation Counter. The lead content of each carcass and all excreta were also measured.

In a subsequent experiment designed to establish whether the effect of Bu_2DTPA was prolonged after discontinuation of treatment, female BCF_1/Anl mice weighing 14-16 g and 48-57 days of age were divided into 11 groups of five to eight mice each. All groups received mouse chow and water *ad libitum* throughout the experiment. Each mouse received an injection *via* tail vein of lead citrate (10 mg Pb/kg, 15 μCi ^{210}Pb/kg) in a 1.25 mg Pb/mL solution. The solution (pH 7.0) was 97.9-99.6% ultrafilterable through cellophane dialysis tubing (Union Carbide Corp.) (LINDENBAUM and WESTFALL 1965). Beginning 3 days after lead injection, treated mice received either two, four, or six intraperitoneal injections of Bu_2DTPA (0.25 mmoles/kg) twice daily (9:35 and 15:35 C.S.T.) for 1, 2, or 3 consecutive days; untreated control mice received intraperitoneal injections of an equal volume (0.15 mL) of 0.9% saline at the same times as the treated mice. Six groups of control mice were sacrificed at 3, 4, 7, 10, 14, and 21 days after lead injection; five groups of mice treated with Bu_2DTPA were sacrificed at 4, 7, 10, 14, and 21 days after lead injection. Mice were killed by exsanguination, and whole blood, brain, spleen, liver, kidneys, lungs, and femurs were taken for analysis of lead as before. The remainder of the body and all excreta were analyzed for determinations of total body retention and excretion.

Statistical comparisons were made using Student's t-test. Total recovery of lead varied from 92% to 94% among different groups of the first experiment and 80% to 87% among different groups of the second experiment.

RESULTS AND DISCUSSION

Comparison of the effects of Bu_2DTPA, DTPA, and EDTA showed that both Bu_2DTPA and DTPA effectively prevented further uptake of lead by the brain after initiation of treatment (Table 1). In contrast, the brains of saline-treated controls continued to accumulate lead; thus, at sacrifice, the amount of lead in the brains of mice treated with Bu_2DTPA was 64% of that measured in saline-treated controls (Table 1). The effects of Bu_2DTPA and DTPA on lead deposition in the brain were not significantly different; EDTA appeared to be less effective. Although therapy did not entirely prevent deposition of lead in the femurs, the mean level of lead at the time of sacrifice for both Bu_2DTPA- and DTPA-treated mice was 65% of that in saline-treated controls. As was found for the brains, EDTA was less effective than DTPA or Bu_2DTPA in preventing lead accumulation within the femurs. In the case of the liver and whole body, Bu_2DTPA was significantly better in removing lead than either DTPA or EDTA (Table 1). In addition, comparison of the three chelators for cumulative urinary or fecal excretion of lead showed that Bu_2DTPA increased fecal lead excretion over that seen in the saline-treated controls, whereas DTPA and EDTA treatment resulted in increased

TABLE 1.

EFFECTS OF THREE CHELATING AGENTS ON REMOVAL OF LEAD FROM MOUSE TISSUES. Lead citrate (30 mg/kg) labeled with ^{210}Pb (81 μCi/kg) was injected i.v., followed at 4, 18, and 24 hours by i.p. injection of either Bu_2DTPA, DTPA, or EDTA (0.25 mmoles/kg as the calcium chelate). Values shown, except for excreta, are means ± standard error.

Group (Numbers of mice)	Hours after Pb	% Injected Lead					
		Brain	Liver	2 Femurs	Whole Body	Urine	Feces
Untreated Controls (4)	4	0.024[b] ±0.001	61.64[c,f] ±3.00	0.58[c,f] ±0.04	90.19[c,f] ±2.93	0.95	1.03
Saline Controls (8)	48	0.044[b,f] ±0.002	35.22 ±1.82	1.68[c,f] ±0.02	74.39[b,e] ±1.85	4.74	14.23
Bu_2DTPA-Treated (5)	48	0.028[b] ±0.001	23.93[a,d] ±1.82	1.10 ±0.04	56.22[a,f] ±1.44	7.46	29.65
DTPA-Treated (5)	48	0.024[b] ±0.002	32.68 ±2.37	1.09 ±0.05	66.10 ±1.21	15.40	12.80
EDTA-Treated (5)	48	0.036 ±0.002	30.85 ±1.98	1.24 ±0.10	64.86 ±2.36	11.47	14.04

[a]Differs from EDTA, $p < 0.05$.
[b]Differs from EDTA, $p < 0.01$.
[c]Differs from EDTA, $p < 0.001$.
[d]Differs from DTPA, $p < 0.05$.
[e]Differs from DTPA, $p < 0.01$.
[f]Differs from DTPA, $p < 0.001$.

urinary lead excretion (Table 1). Thus, Bu_2DTPA, in addition to its action as a chelator, might promote increased excretion of lead via the bilary-fecal routes already utilized by the untreated mammal following lead poisoning (KLAASEN and SHOEMAN 1974).

In the experiment designed to test the duration of effectiveness of these agents, the whole body lead burden of treated animals was not significantly different from that of saline-treated controls on the day after the first two Bu_2DTPA treatments (i.e., 4 days after lead injection); but the reduction was significant after 3 days of twice daily Bu_2DTPA treatments (i.e., 7 days after lead injection) (Fig. 1). Maximum differences in body burden between control and treated mice occurred at 5 days after the last treatment, although the difference was still significant at the last sacrifice time, 16 days after the final Bu_2DTPA treatment (i.e., 21 days after lead injection).

The reductions in femur and whole body lead burdens after Bu_2DTPA therapy generally paralleled one another (Fig. 1). HOFMANN and SEGEWITZ (1975) estimate the total skeletal lead burden to be 20 times the content of one femur. Although femur lead levels may not correspond exactly with changes occurring in the total skeleton (HAMMOND 1971), the correspondence found between whole body and femur burdens suggests that the primary action of the Bu_2DTPA ester was exerted on the skeletal lead burden, as has been reported for EDTA, DTPA, 2,3-dimercaptopropane-1-sulfonate (DMPS), and D-penicillamine (PCA) (HAMMOND et al. 1967; HAMMOND 1971, 1973; and HOFMANN and SEGEWITZ 1975).

Because reversal of neurologic distrubances caused by lead is the primary rationale of this work, it is encouraging that Bu_2DTPA reduced the lead levels in the brain by 10% after only 1 day of treatment (Fig. 1). Maximum differences between control and treated brain levels occurred at 5 and 9 days post-treatment--31% and 33%, respectively; moreover, 16 days after cessation of therapy, treated mice had about 16% less lead in the brain than untreated control animals (Table 2). This result is striking compared to results in blood, kidneys, and liver, where the lead levels of control mice decreased to the levels of treated mice. These results suggest the possibility of achieving even greater reductions in brain levels by a more prolonged treatment regimen.

The primary effect of the Bu_2DTPA treatment regime used here was to hasten excretion from the body of lead that would eventually be excreted without treatment. Except for the brain, and possibly the bone, lead levels in the tissues of the control mice 16 days after the last treatment had decreased to essentially the levels in the mice that had received the Bu_2DTPA treatment. It would seem, however, that shortly after incorporation, when lead burdens are highest in critical soft organs, particularly the brain, accelerated lead decorporation made possible by therapy would be advantageous, especially if such lead deposits

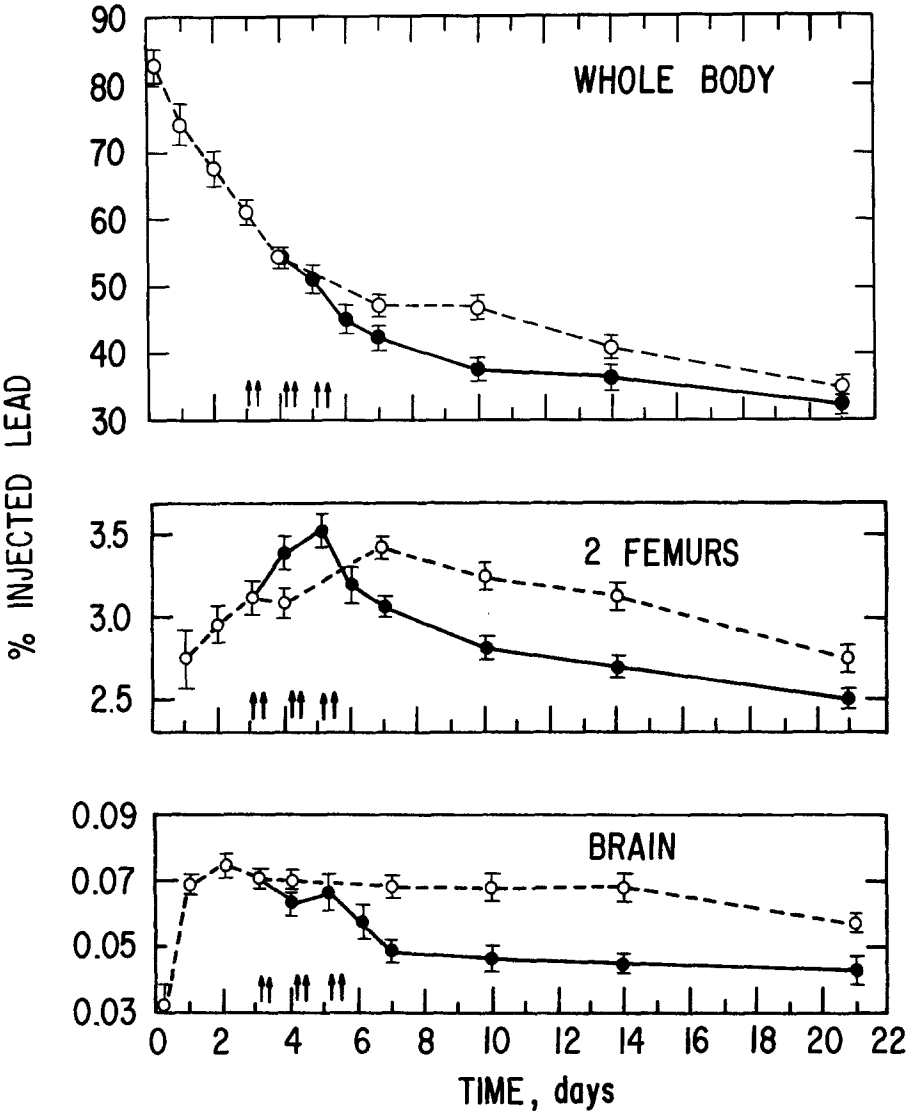

Fig. 1. Mean percent of injected lead (± SE) in whole body, femurs, and brain in treated (●—●) and control (0---0) mice. Immature mice were injected intraperitoneally with Bu_2DTPA (0.25 mmoles/kg) at times shown by arrows.

become increasingly refractory when treatment is delayed, or if irreversible damage results.

Although it is difficult to make comparisons between this study and other lead decorporation studies because of differences in experimental protocols, previous experiments have failed to show significant reductions in one or more critical soft organs (i.e., brain, liver, and kidney) following chelation therapy. In studies on the young rat, a massive dose of 1.0 mmoles/kg of EDTA was incapable of reducing lead levels in the brain (GOLDSTEIN and DIAMOND 1974); nor did 0.28 or 1.1 mmoles/kg PCA (HAMMOND 1971). In the adult rat, however, EDTA or DTPA (1.1 to 1.5 mmoles/kg) given either 4 or 17 days after 7 mg/kg lead significantly reduced the brain burdens (HAMMOND et al. 1967; HAMMOND 1971). The therapeutic doses used by these workers were higher than the level of Bu_2DTPA used in the present work. Furthermore, the actual amount of lead removed from the brain in their experiments was less than that removed in the present experiment because of the lower initial levels. Other studies have shown inconsistant results in critical organs. For example, lead levels in the liver and kidney were reduced in some experiments (HAMMOND et al. 1967; CASTELLINO and ALOJ 1965), but not in others (HAMMOND 1971).

Urinary and fecal lead excretion data (Table 2) show that Bu_2DTPA therapy did not strikingly accelerate the excretion of lead into the urine, as has been previously reported following chelation therapy with EDTA, DTPA, or PCA in man (BRUGSCH et al. 1965; HWANG et al. 1976; STRICKLAND et al. 1976) and with EDTA, DTPA, dimercaptopropanol, PCA, DMPS, or a diazapolyoxa-macrobicyclic complexing agent in rats (ARONSON and HAMMOND 1964; CASTELLINO and ALOJ 1965; HAMMOND 1971, 1973; HOFMANN and SEGEWITZ 1975; BAUDOT et al. 1977). In the present work, treatment with Bu_2DTPA produced a small increase in fecal lead excretion at four time intervals, but urinary excretion was increased at only three (Table 2). It is possible that Bu_2DTPA did not enhance fecal excretion of lead as strikingly in the second experiment as in the first one because treatment was initiated at a time when the level of lead in the liver was much lower than that of the first experiment.

The mobilization of significant quantities of lead by Bu_2DTPA from the brain of the immature mouse, even though initiation of therapy was delayed until 3 days after injection of lead, is the most significant result to emerge from the work reported above. In view of lead-induced neuronal dysfunction in children, this reduction of lead levels in the brains of young mice may be of clinical interest.

ACKNOWLEDGMENTS

The authors thank Drs. Kou-Yi Tserng and John E. Parks for the synthesis of Bu_2DTPA and David P. Peterson for technical assistance. This work was supported by the U.S. Department of Energy.

TABLE 2.

EFFECT OF EXTENDED TREATMENT WITH Bu_2DTPA ON REMOVAL OF LEAD FROM MOUSE TISSUES. Lead citrate (10 mg Pb/kg) labeled with ^{210}Pb (15 μCi/kg) was injected i.v., followed on days 3, 4 and 5 with twice-daily i.p. injections of 0.25 mmoles/kg Bu_2DTPA. Values shown are means ± standard error. Values for excreta are cumulative.

Group (Numbers of mice)	Days after Pb	% Injected Lead/g	% Injected Lead							
		Blood	Brain	Spleen	2 Kidneys	Liver	Lungs	2 Femurs	Urine	Feces
Untreated controls (5)	3	0.31 ±0.02	0.067 ±0.004	4.49 ±0.61	3.04 ±0.40	7.44 ±0.60	2.08 ±0.69	3.13 ±0.11	4.92 ±0.62	21.88 ±2.10
Saline-treated controls (5)	4	0.25 ±0.01	0.070 ±0.002	4.93 ±0.47	1.54 ±0.08	8.62 ±0.73	1.73 ±0.09	3.09 ±0.08	7.07 ±0.12	22.84 ±0.15
Bu_2DTPA-treated (5)	4	0.23 ±0.02	0.063 ±0.003	5.32 ±0.36	1.43 ±0.14	6.43[a] ±0.50	0.99[b] ±0.12	3.40[a] ±0.10	7.15 ±0.22	24.59[b] ±0.19
Saline-treated controls (5)	7	0.20 ±0.005	0.068 ±0.003	3.37 ±0.06	0.57 ±0.02	3.55 ±0.17	1.50 ±0.46	3.42 ±0.03	9.58 ±0.29	27.15 ±0.31
Bu_2DTPA-treated (6)	7	0.16[a] ±0.004	0.049[b] ±0.003	3.23 ±0.27	0.42[c] ±0.02	3.39 ±0.23	0.90 ±0.19	3.07[c] ±0.03	10.30[a] ±0.03	28.62[b] ±0.06
Saline-treated controls (5)	10	0.20 ±0.01	0.068 ±0.004	1.56 ±0.37	0.46 ±0.02	2.44 ±0.27	0.38 ±0.14	3.25 ±0.06	11.04 ±0.13	29.31 ±0.17
Bu_2DTPA-treated (6)	10	0.12[c] ±0.009	0.047[b] ±0.003	1.25 ±0.35	0.34[c] ±0.01	1.76 ±0.24	0.95[b] ±0.24	2.83[b] ±0.06	11.60[a] ±0.11	30.05[a] ±0.08
Saline-treated controls (5)	14	0.12 ±0.005	0.069 ±0.003	0.53 ±0.08	0.37 ±0.01	1.40 ±0.15	0.25 ±0.07	3.14 ±0.08	12.32 ±0.07	30.84 ±0.03
Bu_2DTPA-treated (6)	14	0.10 ±0.004	0.046[c] ±0.002	0.96[a] ±0.11	0.31[c] ±0.004	1.59 ±0.10	0.88[b] ±0.13	2.72[c] ±0.05	13.01[b] ±0.04	31.66[c] ±0.01
Saline-treated controls (5)	21	0.10 ±0.004	0.058 ±0.001	0.17 ±0.04	0.26 ±0.01	0.76 ±0.13	0.34 ±0.09	2.75 ±0.09	14.22 -	33.36 -
Bu_2DTPA-treated (8)	21	0.07[c] ±0.003	0.049[c] ±0.006	0.25 ±0.08	0.24 ±0.01	0.71 ±0.11	0.58[a] ±0.11	2.51[a] ±0.06	14.57 ±0.03	33.51 ±0.10

[a]Differs from saline-treated controls, $p < 0.05$.

[b]Differs from saline-treated controls, $p < 0.01$.

[c]Differs from saline-treated controls, $p < 0.001$.

REFERENCES

ARONSON, A. L. and P. B. HAMMOND.: J. Pharma. Exp. Ther. 146, 241 (1964).

BAUDOT, Ph., M. JACQUE, and M. ROBIN.: Tox. Appl. Pharma. 41, 113 (1977).

BRUGSCH, H. G., N. J. COLOMBO, and L. D. PAGNOTTO.: N. Eng. J. Med. 272, 993 (1965).

CASTELLINO, N. and S. ALOJ.: Br. J. Indust. Med. 22, 172 (1965).

DE LA BURDE, B. and M. S. CHOATE.: J. Ped. 81, 1088 (1972).

FOREMAN, H.: The pharmacology of some useful chelating agents. M. J. Seven and L. A. Johnson ed: Metal-binding in medicine. Philadelphia: J. B. Lippincott Co. (1960).

GOLDSTEIN, G. W. and I. DIAMOND.: Res. Publ. Assoc. Res. Nerv. Ment. Dis. 53, 293 (1974).

GUILMETTE, R. A., J. E. PARKS, and A. LINDENBAUM.: J. Pharm. Sci. (in press).

HAMMOND, P. B., A. L. ARONSON, and W. C. OLSON.: J. Pharma. Exp. Ther. 157, 196 (1967).

HAMMOND, P. B.: Tox. Appl. Pharma. 18, 296 (1971).

HAMMOND, P. B.: Tox. Appl. Pharma. 26, 241 (1973).

HOFMANN, U. and G. SEGEWITZ.: Arch. Tox. 34, 213 (1975).

KLAASEN, C. D. and D. W. SHOEMAN.: Tox. Appl. Pharma. 29, 434 (1974).

LINDENBAUM, A. and W. WESTFALL.: Int. J. Appl. Radiat. Isot. 16, 545 (1965).

MICHAELSON, A. I. and M. W. SAUERHOFF.: Tox. Appl. Pharm. 28, 88 (1974).

MOMČILOVIĆ, B. and K. KOSTIAL.: Envir. Res. 8, 214 (1974).

SILLÉN, L. G. and A. E. MARTELL.: Stability constants of metal-ion complexes. Special Publ. No. 17. London: The Chemical Society (1964).

STRICKLAND, G. T., Y. F. HWANG, N. K. CHANG, and R. Q. BLACKWELL.: Southeast Asian J. Trop. Med. Pub. Hlth. 7, 569 (1976).