Comparison of 3 Chelating Agents in Treatment of Experimental Manganese Poisoning* (24700)

JOAN F. FRIED, ARTHUR LINDENBAUM AND JACK SCHUBERT
(Introduced by A. M. Brues)
Division of Biological and Medical Research, Argonne National Lab., Lemont, Ill.

Ethylenediaminetetraacetic acid, (EDTA), has been tested in treatment of acute manganism of laboratory animals(1,2) and has been recommended for clinical use in the industrial disease(3). It has been suggested that the relative effectiveness of a chelating agent, A, for manganese binding under physiological conditions can be related to the value of the ratio $\frac{K_{MnA}}{K_{CaA}}$(4). This ratio, as shown below, is more favorable in the case of diethylenetriaminepentaacetic acid (DTPA) than for EDTA, indicating that DTPA could be superior to EDTA for treatment of Mn poisoning. In addition, a third compound, ethylene bis (*a* - imino - o - hydroxyphenylacetic acid)† (EBAA) was of interest. While K_{MnA} and K_{CaA} for this compound were not determined, it was known the value of K_{CaA} was considerably smaller than that of EDTA and it was hoped that the net value of $\frac{K_{MnA}}{K_{CaA}}$ would be greater than that for EDTA. Ability of DTPA and EDTA to protect against manganese poisoning was tested in two ways: (a) survival studies on rats given an LD_{90} of manganese gluconate and treated at varying times afterward with either CaEDTA, CaDTPA or saline; and (b) survival studies on rats given the manganese chelates of EDTA and DTPA. The effect of EBAA administration was studied by means of a tissue distribution experiment in which animals were given a tracer dose of Mn^{54} and treated with EBAA, EDTA, or saline, and by a survival experiment in which animals were given an LD_{90} of stable manganese and treated 75 minutes later with EBAA.

Methods. Female Sprague-Dawley rats between 70 and 135 days of age, weighing 190-280 g, were used; animals of equal age were selected for each experimental group. In the survival studies manganese was administered intraperitoneally as a 3.5% solution of manganous gluconate, pH 6.2, at 27 mg/kg as Mn, a dose which had previously been determined to be the 30-day LD_{90} value. The calcium and manganous chelates of EDTA and DTPA were prepared by neutralizing the acid or sodium salt with NaOH, adding calcium or manganous gluconate in 1:1 molar ratio of metal to chelating agent, and adjusting the concentration to 0.16 *M* and the pH to 7.2. CaEDTA and CaDTPA were injected into a tail vein or intraperitoneally in equimolar ratios at levels of from 105 to 2000 mg/kg; doses of these compounds, wherever given, are expressed as the acid form of the chelating agent. MnEDTA and MnDTPA were injected intraperitoneally in doses providing 130 to 260 mg/kg as Mn. EBAA was injected intravenously as the tetrasodium salt at pH 8.5. All survival data given are on a 30-day basis, but surviving animals were kept under observation for 90 days. In the tissue distribution studies, $Mn^{54}Cl_2$ was given intravenously as carrier-free solution to 4 groups of 3 rats each, at a level of 1 μc per rat. These groups were then given intravenous injections of the chelating agents at 1 hr after Mn^{54} injection, and on the following 2 days as follows: (a) EBAA at 80, 40 and 40 mg/kg, (b) CaEDTA in equimolar ratio to this (65, 32.5 and 32.5 mg/kg), (c) CaEDTA at 200 mg/kg per injection

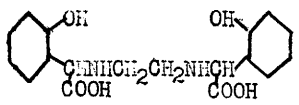
TABLE I.

A	Log K_{MnA}	Log K_{CaA}	Log $\frac{K_{MnA}}{K_{CaA}}$
DTPA(5,6)*	15.1	10.6	4.5, 5.0
EDTA(6,7)	13.5, 13.6, 14.0	10.6	2.9, 3.0, 3.4

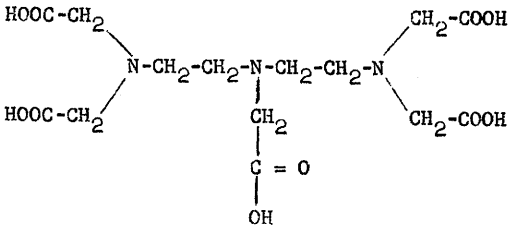
* Values for these constants are in agreement with those obtained from H. Kroll (personal communication, June, 1956).

* This work performed under the auspices of U. S. Atomic Energy Commission.

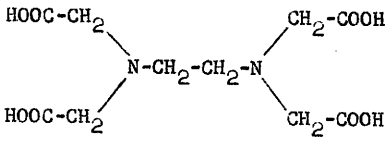
† Supplied through courtesy Geigy Chemical Corp.



EBAA



DTPA



EDTA

and (d) saline in equivalent volume. The rats were sacrificed on the fourth day and the following tissues removed: femurs, brain, pancreas, liver, kidneys and blood. These, and the daily collections of urine and feces, were disintegrated with concentrated nitric acid. The tissues and urines were counted in a NaI (T1) well counter in conjunction with a single channel, pulse height analyzer at the optimum setting for the 0.84-MEV γ-ray. Feces and carcasses were counted in a pressurized ionization chamber designed to permit 100% geometry and sensitive to 0.06 μc Mn^{54}.

Results. Toxicity studies. Intraperitoneal administration of CaDTPA at a level of 2000 mg/kg resulted in 90% survival, while, at a corresponding equimolar level of CaEDTA of 1490 mg/kg, survival was 100% (20 rats per group). However, 70% of the CaDTPA-injected animals at this dose level developed paralysis of the hind limbs on approximately the 6th day after injection; this was of several weeks duration with partial recovery in 2 months. At a slightly lower level (1830 mg/kg) of CaDTPA no deaths or paralyses occurred. Intravenous administration of CaDTPA in amounts up to 250 mg/kg produced no toxic effects. The toxicity data obtained on EBAA were not extensive; however, it was established that 70 mg/kg could safely be given daily for 3 days by the intravenous route.

Survival studies. Equimolar amounts of CaDTPA (158 mg/kg and CaEDTA (118 mg/kg) gave complete protection when injected intravenously as long as 3½ hours after administration of an LD_{90} of manganese gluconate. However, at 5 hours after manganese administration, they were only partially effective (50% survival). Average time to death of non-survivors was longer for the DTPA-treated as compared with the EDTA-treated. In another experiment, the minimum effective dose for complete protection against an LD_{90} of manganese gluconate, when given 1 hour after the manganese, was found to be 115 mg/kg for each of the 2 compounds.

Survival data from rats given the manganese chelates of DTPA and EDTA intraperitoneally are shown in Table II. When manganese is administered as the DTPA chelate, a higher dose may be given without toxic effects than when it is administered as the EDTA chelate.

There was no significant difference in survival of a group of 10 rats given an LD_{90} of manganese gluconate and treated 75 minutes later with 60 mg/kg EBAA intravenously as compared with that of the untreated control group.

Tissue distribution studies. No significant differences were present in distribution of carrier-free Mn among the tissues of the EBAA treated animals as compared with the saline controls (Table III). The route and extent

TABLE II. Survival of Rats Given Manganese Chelates of DTPA and EDTA.

Dose of Mn, mg/kg	Survival, %	
	Mn DTPA	Mn EDTA
130	100 (10)*	100 (10)
162.5	100 (6)	83 (6)
195	100 (17)	70 (17)
225	94 (16)	60 (10)

* No. of animals inj. are in parentheses.

TABLE III. Effect of EBAA and EDTA on Manganese Distribution and Excretion in Rats.

Treatment	Mn^{54} content in % inj. dose*						
	Both femurs	Carcass	Pancreas	Both kidneys	Liver	Urine	Feces
EBAA 80, 40, 40 mg/kg	.25 ±.02	21.8 ±1.3	2.30 ±.27	2.28 ±.35	15.5 ± .8	†	70.1 ±4.83
EDTA 65, 32.5, 32.5 mg/kg	.22 ±.04	21.1 ± .3	2.89 ±.13	1.71 ±.08	17.3 ±1.0	5.87±.96	59.6 ± .8
EDTA 200, 200, 200 mg/kg	.20 ±.03	21.7 ± .5	2.30 ±.15	1.40 ±.17	15.4 ± .9	15.2 ±.2	46.0 ± .1
Saline	.21 ±.02	24.1 ±3.2	2.78 ±.15	2.10 ±.20	14.6 ± .9	†	61.7 ±6.3

* Each value represents mean from 3 rats ± stand. error of mean. Time of sacrifice was 4 days after inj. with Mn.

† Too low to count (less than 2 times background).

of Mn excretion were also unaltered by the EBAA treatment. An equimolar dose of CaEDTA produced a small but significant increase in excretion of Mn in the urine with a corresponding decrease in the feces. The larger dose of CaEDTA promoted a greater shift in the excretion pattern from feces to urine, and lowered the Mn content of the kidneys by about 20%. Neither compound had any effect on quantity of Mn retained in the liver or on total retention of injected dose.

Discussion. The data presented in Table III show little difference in retention of carrier-free Mn between the rats given EDTA and the controls. However, in the case of animals given Mn in large, toxic doses, it has been shown(2) that the liver burden is markedly reduced in EDTA-treated groups. This, together with the reduction in the kidney content, is the basis of the protection afforded by EDTA and DTPA, since animals dying of acute experimental manganese poisoning show marked pathology of liver and kidney.

The primary excretory route of intravenously or intraperitoneally injected manganese is fecal, mainly *via* the bile(8). It has been shown that manganese is excreted in the feces at 2 rates. The faster presumably represents the unbound manganese and the slower represents that portion which becomes bound in tissues, predominantly in the cells of liver, kidney and pancreas(8) and is then slowly released. There is experimental evidence to suggest that EDTA competes successfully with the body tissues for some of the manganese which would otherwise become bound thereby increasing the amount released in the fast component(8). This is accompanied by a shift in excretion of manganese from the feces to an earlier appearance in the urine(8). Presumably DTPA has the same mode of action.

Both CaEDTA and CaDTPA are beneficial in treatment of experimental, acute manganese poisoning. However, there are some indications that CaDTPA is more effective: (a) more manganese may safely be given as the DTPA chelate than as the EDTA chelate, (b) although no significant differences in survival numbers were present when CaDTPA and CaEDTA were given several hours after manganese injection, the time to death of the non-survivors was significantly longer in the case of the CaDTPA treated group, and (c) the dose of CaDTPA needed for protection is smaller, on a molar basis. The titration data obtained in this laboratory(9) indicate that the enhanced effectiveness of CaDTPA is due to greater stability of MnDTPA.

DTPA is far more effective in decreasing the body burden of trivalent and tetravalent elements than is EDTA(10,11,12,13). This is to be expected, since the stability constant ratios $\frac{K_{MA}}{K_{CaA}}$ of DTPA for these metals exceed those of EDTA by factors of 10^2-10^4, whereas in the case of divalent Mn the increase is only of the order of 10.

The failure of EBAA to protect against manganese poisoning is undoubtedly a reflection of too low a value of K_{MnA}/K_{CaA}.

Summary. Three chelating agents of the polyamino polycarboxylic acid type, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, and ethylene bis(α-imino-o-hydroxyphenylacetic acid) have been compared in treatment of acute experimental manganese poisoning. EBAA had no therapeutic value, whereas both EDTA and DTPA provided significant protection to rats given lethal amounts of manganese gluconate. It is shown that DTPA is somewhat more effective than EDTA.

The authors wish to thank Mr. William Westfall and Mrs. Elizabeth Moretti for technical assistance.

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Received December 1, 1958. P.S.E.B.M., 1959, v100.

Coronary Arteriosclerosis and Thrombosis in the Rat.* (24701)

BERNARD C. WEXLER AND BENJAMIN F. MILLER
(With technical assistance of Lionel Staples and Janice Doron)
May Inst. for Medical Research, Cincinnati Jewish Hospital and Depts. of Pathology and Medicine, Univ. of Cincinnati College of Medicine

Severe arteriosclerosis has been produced in female rats (Sprague-Dawley) bred repeatedly, unilaterally nephrectomized, and then given ACTH for 4 to 7 weeks (Wexler and Miller, 1). Lesions involve systemic, cerebral and peripheral arteries. Large, flame-shaped hemorrhages and aneurysms are seen in the aortic arch. Particularly conspicuous upon gross examination are the distended and tortuous coronary vessels. Myocardial infarcts are readily visible. Histologically, a spectrum of early to advanced lesions has been observed in coronary arteries. These findings appear to have a special importance because (a) the rat is ordinarily resistant to development of arteriosclerosis(2-5), (b) these rats were not given fat in their diet and had normal serum cholesterol levels, and (c) the pathology in coronary arteries shows some striking resemblances to that observed in human disease.

Materials and methods. Female rats of the Sprague-Dawley strain were (1) bred repeatedly, (2) subjected to unilateral nephrectomy, and (3) given ⅓ unit of ACTH (gel)/100 g body weight, subcutaneously, 3 times/week, for 4 to 7 weeks. The particular strain of rat can be as important as the other 3 factors in production of satisfactory arteriosclerotic lesions. After nephrectomy, animals were allowed 10 days to recover before administration of ACTH. Animals were housed in air-conditioned quarters and given *ad lib.* a regular commercial rat chow and water. No extra fat or salt was added to the diet. At autopsy, the rats were decapitated, exsanguinated, and tissues intended for microscopic examination quickly removed and fixed in 10% buffered neutral formalin (Lillie). Hematoxylin and

* This work was supported, in part, by a Grant from U.S.P.H.S.