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THE EFFECTS OF DIMERCAPTOSUCCINIC ACID ON THE EXCRETION AND DISTRIBUTION OF MERCURY IN RATS AND MICE TREATED WITH MERCURIC CHLORIDE AND METHYLMERCURY CHLORIDE

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1 All five rats in a group survived if dimercaptosuccinic acid (DMSA), a water soluble derivative of 2,3-dimercaptopropanol (BAL), was given in doses of 10–40 mg/kg intraperitoneally 30 min, 4 and 24 h after administration of 2.4 mg/kg Hg as $HgCl_2$, whereas three out of a group of five died if DMSA was not given. DMSA 20 mg/kg increased urinary excretion and decreased the body burden significantly more than 10 mg/kg DMSA, but further doubling of the dose had only marginal effects.

2 DMSA was able to reduce body burden and increase urinary excretion of Hg when intraperitoneal treatment started eight days after the subcutaneous administration of $HgCl_2$.

3 DMSA was effective in decreasing body burden and the brain concentration of Hg in rats dosed orally with methylmercury (MeHgCl) when intraperitoneal treatment started with 40 mg/kg DMSA 24 h after Hg. Increase in the urinary excretion of mercury was responsible for the decrease in body burden.

4 DMSA was effective when given in the drinking water of rats or mice both against inorganic Hg and MeHgCl. In mice treated intraperitoneally with MeHgCl, DMSA 19.5 μg/ml in the drinking water caused a significant decrease in the body burden and increase in the excretion of Hg.

5 DMSA was about four times more efficient than D-penicillamine in decreasing the body burden of Hg. As their toxicity is in the same range, the higher efficiency of DMSA offers a larger margin of safety for the mobilization of Hg.

Introduction

After the discovery that 2,3-dimercaptopropanol (BAL) is effective against $HgCl_2$ intoxication both in experimental animals (Braun, Lusky & Calvery, 1946) and in man (Longcope & Leutscher, 1949), the search has never stopped for a more efficient and less toxic chelating agent. This effort led to the discovery that D-penicillamine (Aposhian, 1958) and N-acetyl-DL-penicillamine (Aposhian & Aposhian, 1959) were both effective antidotes against $HgCl_2$ in animal experiments, though their efficiency in man for the treatment of $HgCl_2$ intoxication is not consistent (Kazantzis, Schiller, Asscher & Drew, 1962; Teisinger & Srbova, 1964; Javett & Kaplan, 1968; Kark, Poskanzer, Bullock & Boylen, 1971).

The modification of BAL led to the synthesis of 2,3,-dimercaptopropanol-sulphonate which under the name of Unithiol is used in the USSR and has proved to be an effective antidote against $HgCl_2$ (Belonozhko, 1958). Another derivative of BAL: meso-dimercaptosuccinic acid (DMSA) (HOOC-CHSH-CHSH-COOH) was used recently for the mobilization of Hg in mice and guinea-pigs both after the administration of $HgCl_2$ and MeHgBr (Friedheim & Corvi, 1975). After a single dose of $HgCl_2$ or MeHgBr two daily doses of 100 mg/kg DMSA given intraperitoneally decreased the kidney, liver and the brain content of mercury in mice. In guinea-pigs the same effect was tested and confirmed with 250 to 1000 mg/kg DMSA. Equal doses of D-penicillamine were less effective, though the LD_{50} for D-penicillamine (Friedrich & Zimmermann, 1975) and for DMSA (Friedheim & Corvi, 1975) is the same: between 3.0 and 4.0 g/kg. The present study is an extension of Friedheim & Corvi's work. Significantly smaller doses of DMSA were used and their effect on the survival of rats after the administration of $HgCl_2$ and on the body burden and the excretion of Hg was followed in rats and mice, both after the administration of $HgCl_2$ and MeHgCl. Because of the convenience of oral administration, the effect of DMSA was also tested when it was administered in the drinking water.

Methods

White male rats (Porton-Wistar strain) of approximately 200 g body weight and white male mice

(LACA strain) of approximately 20 g body weight were used.

Hg was injected either as $HgCl_2$ (Analar, B.D.H. Laboratory Chemicals, Poole) or MeHgCl (K & K Laboratories, Plainview, N.Y.) labelled with $^{203}HgCl$ or $Me^{203}HgCl$ (Radiochemical Centre, Amersham). $Me^{203}HgCl$ supplied by the Radiochemical Centre was extracted from the aqueous solution into carbon tetrachloride which was subsequently evaporated and the residue dissolved in water. No more than 2% of the total Hg in the injection solution of MeHgCl was inorganic as estimated by the method of Magos & Clarkson (1972).

Doses expressed in Hg, volumes of the injection solutions and specific activities were as follows: in experiments with $HgCl_2$ rats were injected intraperitoneally with 2.4 mg/kg in a volume of 3.2 ml/kg (sp. act. 0.12 μCi/mg) or subcutaneously with 0.5 mg/kg in a volume of 2.0 ml/kg (sp. act. 1.0 μCi/mg); MeHgCl was given to rats by oral cannulation in a volume of 2.0 ml/kg either in a single dose of 3.36 mg/kg (sp. act. 0.26 μCi/mg) or six daily doses (with two days interval between the fourth and the fifth dose) of 6.0 mg/kg (sp. act. 0.1 μCi/mg).

Mice were given both $HgCl_2$ (sp. act. 40 μCi/mg) and MeHgCl (sp. act. 15–25 μCi/mg) in a dose of 0.5 mg/kg intraperitoneally in a volume of 10 ml saline/kg. DMSA (supplied by E.A.H. Friedheim, Geneva) was given to rats either intraperitoneally in a volume of 5 ml/kg in saline (0.9% w/v NaCl solution) or in the drinking water containing 0.5% disodium edetate (EDTA). Controls were injected with saline or given 0.5% EDTA in the drinking water. The volume of drinking water was 30 ml per rat, and almost all was consumed by the animals. Mice were given DMSA in the drinking water. Five mice drank approximately 20 ml per day from the 25 ml drinking water supplied. In some experiments mice were given D-penicillamine 2.5 mg/ml (Dista Products Ltd., Liverpool) instead of DMSA. When urine and faeces was collected two rats were placed in one metabolic cage. Faeces and urine were separated according to Östlund (1969) from five mice kept in one cage.

Details of experimental schedules are given in the Tables.

Radioactive measurements

Live animals placed in a $14 \times 6 \times 6.5$ cm plexiglass box were counted with an efficiency of 7.7% between two NaI crystal pairs (No. N656, EKCO Electronics Ltd., Southend-on-Sea, Essex) facing each other. Faeces and urine samples, blood and liver, in beakers, were counted in the same system. Kidneys and spleen and in some experiments blood were counted in a well-shaped NaI crystal with an efficiency of 31%. Injection standards in 1 ml acid permanganate solution were counted and Hg concentrations in organs and in whole animals were calculated after corrections for geometrical differences by relating their radioactivities to standards.

Mathematical calculations

Half times were calculated from the equation

$$A = A_0 \times e^{-bt}$$

where A_0 is the injected dose, A is the body burden at time t (in days) by the use of the weighted least mean square curves of best fit essentially as described by

Table 1 The effect of dimercaptosuccinic acid (DMSA) on survival, body burden and excretion of Hg in male rats injected subcutaneously with 2.4 mg/kg Hg as $HgCl_2$

				% of initial body burden				
Dose of DMSA (mg/kg)	Decrease in initial body burden (mean ± s.e. mean)			Urinary excretion		Faecal excretion		6 day
	24 h	48 h	6 day	0–24 h	24–48 h	0–24 h	24–48 h	mortality
—	10.0 ± 1.16 (n=5)	20.1 ± 3.04 (n=5)	52.1;54.9 (n=2)	7.5	0.6	4.2	11.8	3/5
10	27.0 ± 2.99* (n=5)	38.0 ± 2.54* (n=5)	61.4 ± 2.80* (n=5)	17.5	3.0	5.0	10.6	0/5
20	37.0 ± 1.61 (n=5)	48.8 ± 3.99 (n=5)	72.4 ± 2.34 (n=5)	26.4	10.0	4.1	14.1	0/5
40	36.0 ± 2.40 (n=5)	54.7 ± 3.17 (n=5)	76.8 ± 1.85 (n=5)	28.0	14.0	5.6	12.0	0/5

Dimercaptosuccinic acid was given intraperitoneally in saline 30 min, 4 h and 24 h after the administration of Hg in a volume of 5 ml/kg. Control animals were given the same volume of saline. When faeces and urine were collected, two animals shared one metabolic cage and excreta were collected from four animals per group.
* Significantly different ($P < 0.05$) from groups given higher doses of DMSA with one exception: at 48 h the 10 mg/kg group differs significantly only from the 40 mg/kg group.

Deming (1964) using a programmable desk type calculating machine (Hawlet-Packard 9810A calculator). Statistical differences were calculated by Student's t test.

Results

Table 1 shows that three of the five rats injected subcutaneously with 2.4 mg/kg Hg as $HgCl_2$ died within six days. None of the animals treated with DMSA died. Compared with the controls the body burden decreased 100% more and urinary excretion of Hg increased at least 2.5 times in the first 48 h after Hg in rats dosed with 10 mg/kg DMSA. Treatment with 20 mg/kg DMSA resulted in a further decrease in the body burden and increase in the urinary excretion of Hg. A further increase of the dose of DMSA did not affect the body burden of Hg. Faecal excretion remained unchanged by DMSA.

Table 2 shows that DMSA is able to mobilize Hg eight days after the injection of 0.5 mg/kg Hg as

Table 2 The effect of three doses of 20 mg/kg dimercaptosuccinic acid (DMSA) given intraperitoneally eight days, eight days + 6 h and nine days after the subcutaneous administration of 0.5 mg/kg Hg as $HgCl_2$ on the excretion, body burden and organ contents of Hg in male rats

	No. of rats per group	*Hg in % of the pretreatment body burden (mean ± s.e. mean) in control*	*in DMSA treated*
Excretion			
Urine 0–24 h	10	1.8 ±0.14	9.3 ±0.44*
24–48 h	10	0.9 ±0.12	2.4 ±0.27*
Faeces 0–24 h	10	1.5 ±0.12	2.3 ±0.15*
24–48 h	10	1.1 ±0.09	1.5 ±0.06
Hg Content			
Whole body	10	92.9 ±1.38	87.0 ±1.07*
Kidneys	10	80.1 ±1.70	74.8 ±0.88*
Blood	10	0.47 ±0.03	0.45 ±0.03
Liver	10	1.5 ±0.13	1.7 ±0.10
Spleen	10	0.081 ±0.007	0.082 ±0.004

Animals were killed 48 h after the first DMSA injection. Urine and faecal samples were obtained from two animals. Calculation for blood mercury was based on the assumption that 7% of the body weight is blood.
* Significantly different from the control ($P < 0.05$).

Table 3 The effect of three doses of 40 mg/kg dimercaptosuccinic acid (DMSA) given intraperitoneally on the excretion, body burden and organ contents of Hg in male rats dosed with 3.36 mg/kg Hg as MeHgCl by oral cannulation in a volume of 2 ml/kg

	Hg in % of the dose (mean ± s.e. mean) in control	*in DMSA-treated*
Excretion after first DMSA injection		
Urine 0–24 h	0.8	20.3
24–48 h	1.5	7.4
Faeces 0–24 h	2.9	2.1
24–48 h	3.5	2.9
Hg content		
Whole body	89.1 ±2.38	62.7 ±1.58*
Brain	0.31 ±0.011	0.22 ±0.010*
Kidneys	3.2 ±0.10	2.2 ±0.15*
Blood	41.5 ±1.00	28.6 ±1.15*
Liver	7.3 ±0.32	5.1 ±0.23*

DMSA was given 24, 30 and 48 h after mercury. Animals were killed 48 h after the first DMSA injection. There were four animals per group. Values for urine and faeces are the mean of two samples obtained from four animals.
* Significantly different ($P < 0.05$) from the control.

$HgCl_2$. The effect on body burden and the urinary excretion of Hg was significant. Faecal excretion of Hg in the first 24 h also increased. At the end of the 48 h treatment period, the concentration of Hg in the blood and spleen remained the same as in the controls but DMSA significantly decreased the kidney burden of Hg.

Table 3 shows that DMSA given 24–48 h after the *per os* administration of 3.36 mg/kg Hg as MeHgCl decreased the body burden and increased the urinary excretion of Hg significantly and decreased the concentration of Hg in every organ tested including the brain.

Table 4 shows that DMSA was effective in decreasing the body burden and the concentration of Hg in brain, blood, liver and kidneys when DMSA was given in the drinking water to rats pretreated repeatedly with MeHgCl. DMSA given in the drinking water of mice treated intraperitoneally with $HgCl_2$ (Table 5) or with MeHgCl (Table 6) decreased the biological half time and increased the urinary excretion of Hg. Even 19.5 μg/ml DMSA was effective. The effect on faecal excretion was not consistent. The urinary excretion of Hg was always increased significantly by DMSA and both urinary excretion and decrease in body burden showed a dose-dependence.

D-Penicillamine 2.5 mg/ml in the drinking water of mice treated with the same dose of MeHgCl resulted in a biological half time of approximately 2.2 days. Animals excreted 16.9 ± 1.4% daily of the body burden in the urine and 4.8 ± 0.26% daily in the faeces.

Discussion

The present experiments confirmed the effectiveness of DMSA in decreasing the body burden of Hg. This effect is more pronounced after the administration of MeHgCl than after inorganic Hg. Moreover, it has been shown that DMSA exerts its protective effect not only when it was given shortly after the administration of Hg compounds but also if the treatment started after a considerable delay and by significantly lower doses than used by Friedheim & Corvi (1975). It was

Table 4 Effect of dimercaptosuccinic acid (DMSA) given in the drinking water (2.5 mg/ml) three days after the last of the six daily doses of methylmercury (MeHgCl) in male rats.

		Hg concentration in μg Hg/g (mean ± s.e. mean)					
Treatment	*No. of rats*	*Whole body 3 days after Hg*	*Whole body 9 days after Hg*	*Brain*	*Blood*	*Kidneys*	*Liver*
—	5	27.5 ± 0.69	20.1 ± 1.07	8.7 ± 0.46	99.3 ± 8.34	95.9 ± 7.34	22.5 ± 1.53
DMSA	4	26.8 ± 0.69	10.1 ± 0.15*	4.0 ± 0.17*	28.0 ± 1.22*	37.8 ± 2.3*	6.6 ± 0.69*

MeHgCl was given *per os* in a dose of 6.0 mg/kg Hg. Treatment with DMSA lasted for three days. Animals were killed nine days after the last dose of MeHgCl that is three days after the end of the DMSA treatment.
* Significantly different ($P < 0.05$) from the control.

Table 5 Effect of dimercaptosuccinic acid (DMSA) on the half life and excretion of Hg in male mice

DMSA in the drinking water (mg/ml)	*Half time (days)*		*Daily excretion in % of body burden (mean ± s.e. mean)*	
	Mean	*Limits of s.e. mean*	*in urine*	*in faeces*
—	3.51 $n=5$	3.27–3.79	7.52 ± 1.85 $n=4$	8.67 ± 0.80 $n=4$
0.156	1.99*† $n=5$	1.94–2.04	11.08 ± 1.17 $n=4$	14.77 ± 1.03* $n=4$
0.625	1.53* $n=5$	1.50–1.56	18.00 ± 2.87* $n=4$	14.65 ± 0.88* $n=4$

$HgCl_2$ was given intraperitoneally in a dose of 0.5 mg Hg/kg, DMSA was given in the drinking water. Body burden was counted for five days and excreta collected for four days after treatment with Hg. Urine or faecal samples were collected from five mice.
* Significantly different ($P < 0.05$) from the control; † significantly different from the higher dose groups.

effective not only after parenteral administration but also if it was given in the drinking water. In the drinking water of mice a concentration as low as 19.5 μg/ml which corresponds to a dose of 4.0 mg/kg had a significant effect on the half time of the body burden after the administration of MeHgCl. D-Penicillamine given in similar experimental circumstances was at least four times less effective than DMSA (Magos & Stoytchev, 1969).

The decrease in body burden paralleled the decrease in the mercury concentration in the critical organs, in the kidneys, and in the brain in the case of MeHgCl. As Table 2 shows, the amount of Hg removed from the kidneys of inorganic Hg-treated rats was completely excreted and not redistributed in the body. In the MeHgCl experiments the kidney in relation to body burden contained 25 times less Hg; the loss of Hg was not restricted to one organ but to every tissue tested.

DMSA both in sublimate- and MeHgCl-treated rats decreased the body burden mainly by increasing the urinary excretion of Hg. Faecal excretion of Hg was either increased or not affected by DMSA. Swensson & Ulfvarson (1967) observed the same effect with BAL. However, when data in Table 2 are compared with experiments carried out in similar circumstances (Magos & Stoytchev, 1969) it is clear that, on a molar basis DMSA was slightly more efficient in increasing the urinary excretion of Hg than BAL and at least four times as efficient as D-penicillamine but unlike BAL and D-penicillamine, DMSA did not increase the blood concentration of Hg. A redistribution caused by BAL leading to higher Hg concentration in the brain in methylmercury-treated rats (Berlin, Jerksell & Nordberg, 1965) is the major contraindication in the use of BAL to decrease the body burden of methylmercury. Another point which needs consideration is that the toxicity of BAL is high, the LD_{50} for

Table 6 Effect of dimercaptosuccinic acid (DMSA) on the half life and excretion of methylmercury (MeHgCl) in male mice

	Treatment with methylmercury			
DMSA in drinking water (mg/ml)	*Half time (days)*		*Daily excretion in % of the body burden (mean ± s.e. mean)*	
	Mean	*Limits of s.e. mean*	*in urine*	*in faeces*
—	10.88 $n=20$	10.47 – 11.33	1.94 ± 0.19 $n=16$	3.89 ± 0.24 $n=6$
0.0120	6.04*† $n=5$	5.77 – 6.35	2.95 ± 0.30*† $n=4$	5.50 ± 0.52* $n=4$
0.039	4.29*† $n=5$	4.08 – 4.52	9.28 ± 0.69* $n=4$	4.37 ± 0.38 $n=4$
0.078	2.64*† $n=5$	2.57 – 2.72	10.63 ± 0.97*† $n=4$	7.10 ± 1.20* $n=4$
0.1562	2.42*† $n=5$	2.40 – 2.43	14.40 ± 1.27* $n=4$	4.25 ± 0.15 $n=4$
0.313	1.84*† $n=10$	1.75 – 1.94	15.30 ± 1.18*† $n=8$	4.34 ± 0.49 $n=8$
0.625	1.21*† $n=10$	1.15 – 1.28	23.50 ± 1.92* $n=8$	5.21 ± 0.70 $n=8$
1.25	0.784* $n=9$	0.782 – 0.786	23.27 ± 1.89* $n=4$	7.25 ± 1.40* $n=4$
2.50	0.735* $n=5$	0.690 – 0.786	27.23 ± 1.81*† $n=4$	5.53 ± 0.94 $n=4$
5.00	0.638* $n=5$	0.613 – 0.665	43.03 ± 3.39* $n=4$	5.37 ± 1.579 $n=4$

MeHgCl was given intraperitoneally in a dose of 0.5 mg Hg/kg, DMSA was given in the drinking water. Body burden was counted and excreta collected for four days after treatment with methylmercury. Every urine and faeces sample was collected from five mice.

* Significantly different ($P<0.05$) from the control; † significantly different from the next higher dose; for faecal excretion only significant difference is given between control and treated groups.

mice being 100 mg/kg (Durlacher, Bunting, Harrison, Ordway & Albrink, 1946).

Summarizing the advantage of DMSA, the following points seem particularly significant. Firstly DMSA, like D-penicillamine and Unithiol, is a water-soluble compound which makes administration convenient. Secondly its toxicity is in the same range as that of D-penicillamine and it is approximately 35 times less toxic than BAL. The LD_{50} for Unithiol in mice in 1270 mg/kg (Kostygov, 1958) which makes Unithiol approximately three times more toxic than DMSA. Thirdly, compared with D-penicillamine the efficiency of DMSA on the basis of mg/kg is four times higher in decreasing the body burden and increasing the urinary excretion of Hg. Consequently in clinical trials lower doses of DMSA may be used than the usual dose of D-penicillamine.

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References

APOSHIAN, H.V. (1958). Protection by D-penicillamine against the lethal effects of mercury chloride. *Science*, **128**, 93.

APOSHIAN, H.V. & APOSHIAN, M.M. (1959). N-acetyl-DL-penicillamine, a new oral protective agent against the lethal effects of mercuric chloride. *J. Pharmac. exp. Ther.*, **126**, 131–135.

BELONOZHKO, G.A. (1958). Therapeutic action of Unithiol in poisoning with inorganic mercury compounds. *Farmakol. i Toxikol.*, **21**, No. 3, 69–73.

BERLIN, M., JERKSELL, L.-G. & NORDBERG, G. (1965). Accelerated uptake of mercury by brain caused by 2,3-dimercaptopropanol (BAL) after injection into the mouse of a methylmercury compound. *Acta pharmac. Tox.*, **23**, 312–320.

BRAUN, H.A., LUSKY, L.M. & CALVERY, H.O. (1946). The efficacy of 2,3-dimercaptopropanol (BAL) in the therapy of poisoning by compounds of antimony, bismuth, chromium, mercury and nickel. *J. Pharmac. exp. Ther.*, **87**, suppl. 119–125.

DEMING, W.E. (1964). *Statistical Adjustment of Data*. New York: Dover Publications.

DURLACHER, S.H., BUNTING, H., HARRISON, H.E. ORDWAY, N.K. & ALBRINK, W.S. (1946). The toxicological action of 2,3-dimercaptopropanol (BAL). *J. Pharmac. exp. Ther.*, **87**, suppl. 28–32.

FRIEDHEIM, E. & CORVI, C. (1975). Meso-dimercaptosuccinic acid, a chelating agent for the treatment of mercury poisoning. *J. Pharm. Pharmac.*, **37**, 624–626.

FRIEDRICH, L. & ZIMMERMANN, F. (1975). Zur Pharmakologie von Penicillamin. *Arzneimittel-Forsch.*, **25**, 172–168.

JAVETT, S.N. & KAPLAN, G. (1968). Acrodynia treated with D-penicillamine. *Amer. J. Dis. Child.*, **115**, 71–73.

KARK, R.A., POSKANZER, D.C., BULLOCK, J.D. & BOYLEN, G. (1971). Mercury poisoning and its treatment with N-acetyl-α-penicillamine. *New Engl. J. Med.*, **285**, 10–16.

KAZANTZIS, G., SCHILLER, K.F.R., ASSCHER, A.W. & DREW, R.G. (1962). Albuminuria and the nephrotic syndrome following exposure to mercury and its compounds. *Quart. J. Med.*, **28**, 403–418.

KOSTYGOV, N.M. (1958). The antidotal action of mercaptosuccinic acid and Unithiol against mercury. *Farmakol. i Toxickol.*, **21**, No. 3, 64–69.

LONGCOPE, W.T. & LEUTSCHER, J.A. (1949). The use of BAL (British Anti-Lewisite) in the treatment of the injurious effects of arsenic, mercury and other metallic poisons. *Ann. Intern. Med.*, **31**, 545–554.

MAGOS, L. & CLARKSON, T.W. (1972). Atomic absorption determination of total, inorganic, and organic mercury in blood. *J. Assoc. Off. Anal. Chem.*, **55**, 966–971.

MAGOS, L. & STOYTCHEV, Ts. (1969). Combined effect of sodium maleate and some thiol compounds on mercury excretion and redistribution in rats. *Br. J. Pharmac.*, **35**, 121–126.

ÖSTLUND, K. (1969). Studies on the metabolism of methyl mercury and dimethylmercury in mice. *Acta pharmac. tox.*, **27**, suppl. 1.

SWENSSON, A. & ULFVARSON, V. (1967). Experiments with different antidotes in acute poisoning by different mercury compounds. *Int. Arch. Gewerbepath. Gewerbehyg.*, **24**, 12–50.

TEISINGER, J. & SRBOVA, J. (1964). Effect of D-penicillamine on the urinary excretion of mercury and lead. *Prac. Lek.*, **16**, 433–435.

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