Chelation in Metal Intoxication XV: Influence of Dimercaptopropane Sulphonate (DMPS) on Lead Poisoned Rats with Normal or Damaged Kidneys

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Abstract: The efficacy of 2, 3 dimercaptopropane 1, sulphonate (DMPS) to enhance urinary excretion of lead, reduce the concentration of Pb in blood, brain, kidneys and liver and restore Pb induced alterations in the activities of renal and blood enzymes and brain biogenic amines in rats with normal or experimentally damaged kidneys, was investigated. The acute renal damage was induced by uranyl acetate prior to treatment with DMPS. The chelating agent was effective in reducing body burden of Pb by enhancing its excretion and in restoring biological alterations, more markedly in animals with normal kidneys than in those with acutely damaged kidneys. However, the increased brain concentration of Pb and altered levels of brain catecholamines remained unaffected on treatment with DMPS.

Key words: DMPS—Lead toxicity—Uranyl acetate—Enzyme—Kidney—Rat

INTRODUCTION

Kidney is the target organ for lead effects. The investigations suggest the presence of kidney damage, as measured by blood urea nitrogen (BUN), creatinine and clearance studies in workers with a history of lead exposure and with blood lead generally in the range of 60–80 $\mu\text{g}/100\text{ ml}$.^{1–4)} Cooper and Gaffey⁵⁾ found an increased risk of death from chronic and unspecified nephritis among workers exposed to lead.

The experience with the treatment of lead poisoning by some well known heavy metal chelators such as calcium versenate (Ca EDTA) and 2,3-dimercaptopropanol (BAL) has raised serious doubts about their prophylactic use in humans particularly with existing renal dysfunction owing to their undesirable effects. 2,3-Dimercaptopropane 1, sulphonate (DMPS, Unithiol), a water soluble derivative of BAL has been suggested to be an effective alternate for the treatment of lead poisoning.⁶⁾ However, the efficacy and safety of the compound might greatly depend on the renal condition. With a view to ascertain the suitability of DMPS for the treatment

of lead poisoning in individuals with renal dysfunction, caused either by lead itself or some other factors, the effect of DMPS on lead intoxicated rats with normal and experimentally damaged kidneys was investigated.

MATERIALS AND METHODS

Male albino rats weighing 150 ± 10 g of ITRC colony maintained on standard pellet diet (Hindustan Lever Ltd., India) and water *ad libitum* were administered 10 mg/kg, Pb as Pb (OCOCH₃)₂ · 3H₂O dissolved in water, orally for four weeks. The animals receiving water alone served as normal control. Lead treated rats were divided equally into two groups. The animals of group I were given single injection of uranyl acetate (3 mg/kg, s.c.) and those of group II received an equivalent amount of sodium acetate. Six animals from each group and 6 normal controls were kept individually in metabolic cages for 24 hr urine collection in ice cold tubes for 3 days. All the normal controls and six animals from groups I and II were decapitated on the 4th day. Kidneys, liver and brain were removed and blood was collected from the heart in heparinized tubes.

Half of the remaining rats in groups I and II were administered DMPS (0.3 m mole (63 mg)/kg, i.p. 2 doses 8 hr apart) dissolved in normal saline and the other half received an equal volume of saline alone. Six animals, after each treatment, were kept in metabolic cages for 24 hr urine collection for 4 days. Tissue and blood were collected thereafter.

Standard procedures were used to determine the activities of lactic dehydrogenase (LDH)⁷⁾, glutamic oxalacetic transaminase (GOT)⁸⁾ and alkaline phosphatase (ALP)⁹⁾ in urine and kidney homogenate (10%, w/v). The excretion of δ -aminolevulinic acid (δ -ALA) was measured in the last urine sample using the method of Davis *et al.*¹⁰⁾ The activity of blood δ -aminolevulinic acid dehydratase (δ -ALAD)¹¹⁾ hemoglobin (Hb),¹²⁾ packed cell volume (PCV)¹³⁾ and Zinc protoporphyrin (ZPP)¹⁴⁾ levels were measured immediately after collection. The levels of dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) were estimated in brain.¹⁵⁾

Lead was estimated in urine,¹⁶⁾ blood,¹⁷⁾ kidneys, liver and brain¹⁸⁾ following wet acid digestion using atomic absorption spectrophotometer (Perkin Elmer 5,000).

RESULTS

Exposure to Pb for four weeks significantly increased blood, kidney, liver and brain concentrations of Pb, blood ZPP, urinary δ -ALA, inhibited the activities of blood δ -ALAD, renal LDH, GOT, ALP and decreased the blood Hb. Lead also decreased the levels of brain DA and 5-HT and increased that of brain NE (Table 1). The urinary excretion of Pb increased and that of LDH, GOT and

Table 1. Effect of uranyl acetate on Pb induced alterations in rats

Parameters	Normal control	Administration after Pb exposure	
		Sodium acetate	Uranyl acetate
<i>Blood</i>			
Pb ($\mu\text{g}/100\text{ ml}$)	12.6 $\pm$ 0.90	116.3 $\pm$ 10.10 ^{a)}	131.3 $\pm$ 12.10 ^{a)}
δ -ALAD (μ mole δ -ALA/min/ 1-erythrocyte)	4.6 $\pm$ 0.30	1.9 $\pm$ 0.20 ^{a)}	0.8 $\pm$ 0.30 ^{a)} **
Hb (g/100 ml)	13.4 $\pm$ 0.70	10.1 $\pm$ 0.30 ^{b)}	9.4 $\pm$ 0.50 ^{a)}
PCV (% volume)	44.7 $\pm$ 0.40	45.0 $\pm$ 1.10	42.8 $\pm$ 1.70
ZPP (μg ZPP/g, Hb)	2.1 $\pm$ 0.40	7.1 $\pm$ 0.70 ^{a)}	10.3 $\pm$ 0.30 ^{a)} **
<i>Urine</i>			
δ -ALA (mg/100 ml)	0.1 $\pm$ 0.01	0.3 $\pm$ 0.01 ^{a)}	0.3 $\pm$ 0.04 ^{a)}
<i>Kidney</i>			
Pb ($\mu\text{g}/\text{g}$, fresh tissue)	4.4 $\pm$ 0.80	16.7 $\pm$ 1.10 ^{a)}	25.3 $\pm$ 1.90 ^{a)} **
LDH (μ mole NADH oxidized/ min/mg protein)	3.8 $\pm$ 0.50	2.1 $\pm$ 0.30 ^{b)}	1.1 $\pm$ 0.20 ^{a)} **
GOT (n mole hydrazone formed/min/mg protein)	28.3 $\pm$ 1.30	24.7 $\pm$ 1.30 ^{c)}	17.3 $\pm$ 1.90 ^{a)} **
ALP (μ mole phenol liberated/min/mg protein)	1.4 $\pm$ 0.03	0.8 $\pm$ 0.04 ^{a)}	0.4 $\pm$ 0.09 ^{a)} **
<i>Liver</i>			
Pb ($\mu\text{g}/\text{g}$, fresh tissue)	4.7 $\pm$ 0.90	30.1 $\pm$ 1.70 ^{a)}	31.3 $\pm$ 1.90 ^{a)}
<i>Brain</i>			
Pb ($\mu\text{g}/\text{g}$, fresh tissue)	0.5 $\pm$ 0.03	3.1 $\pm$ 0.04 ^{a)}	3.1 $\pm$ 0.07 ^{a)}
DA ($\mu\text{g}/\text{g}$, fresh tissue)	0.41 $\pm$ 0.02	0.36 $\pm$ 0.01 ^{c)}	0.35 $\pm$ 0.20 ^{c)}
NE ($\mu\text{g}/\text{g}$, fresh tissue)	0.24 $\pm$ 0.01	0.28 $\pm$ 0.02 ^{c)}	0.27 $\pm$ 0.00 ^{c)}
5-HT ($\mu\text{g}/\text{g}$, fresh tissue)	0.48 $\pm$ 0.01	0.04 $\pm$ 0.00 ^{c)}	0.43 $\pm$ 0.01 ^{c)}

Each figure is mean $\pm$ S.E. of 6 samples

^{a)} $p < 0.001$, ^{b)} $p < 0.01$, ^{c)} $p < 0.05$, versus normal control; ** $p < 0.01$ versus sodium acetate administered group, as evaluated by the student's 't' test.

ALP remained within normal range at the end of four weeks of Pb exposure (Fig. 1). However, single s.c. injection of uranyl acetate enhanced the urinary excretion of LDH, GOT and ALP, further increased the concentration of Pb and inhibited the activities of these enzymes in kidneys showing acute renal damage (Fig. 1, Table 1). Uranyl acetate also enhanced the Pb induced inhibition in the activity of blood δ -ALAD and elevation of blood ZPP level (Table 1).

DMPS was effective in enhancing urinary excretion of Pb and in reducing urinary δ -ALA. The effects were more marked in animals with normal kidneys than in those with damaged kidneys (Fig.2 and 3). The level of renal Pb increased and that of hepatic Pb decreased with time after cessation of Pb exposure without treatment with DMPS. However, DMPS lowered blood, renal, and hepatic Pb levels; the renal depletion being significantly more marked in animals with normal kidneys (Table 2). The treatment with DMPS restored lead induced inhibition in the activity of blood δ -ALA-D and elevation of blood ZPP more effectively in animals with normal kidneys (Table 3).

The chelating agent however, failed to restore the lead induced alterations in

Fig. 1. Effect of uranyl acetate on urinary excretions of Pb and enzymes in Pb preexposed rats.

Each bar represents mean $\pm$ S.E. of 6 values. Horizontal lines represent normal control (mean $\pm$ S.E. of 6 values) a) $p < 0.001$, b) $p < 0.01$, versus normal control *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ versus Pb-sodium acetate administered group as evaluated by the student's 't' test.

Fig. 2. Effect of DMPS on urinary excretion of Pb in Pb poisoned rats.

Each bar represents mean $\pm$ S.E. of 6 values. Horizontal lines represent respective saline control. a) $p < 0.001$, b) $p < 0.01$ versus saline control; * $p < 0.05$ versus sodium acetate administered group as evaluated by the students's 't' test.

Fig. 3. Effect of DMPS on urinary excretion of δ -ALA in Pb poisoned rats on the 4th day.

Each bar represents mean $\pm$ S.E. of 6 values. a) $p < 0.001$ versus saline control; * $p < 0.05$ versus Pb-sodium acetate administered group as evaluated by the student's 't' test.

Table 2. Effect of DMPS on the blood and tissue levels of Pb in lead poisoned rats

	Pb-sodium acetate		Pb-uranyl acetate	
	saline	DMPS	saline	DMPS
Blood†	103.79 $\pm$ 8.15 (5)	80.46 $\pm$ 6.74 ^{c)} (5)	123.24 $\pm$ 6.58 (5)	82.43 $\pm$ 10.11 ^{c)} (5)
Kidney††	26.84 $\pm$ 1.19 (5)	8.22 $\pm$ 1.06 ^{a)} (5)	29.61 $\pm$ 1.92 (5)	13.99 $\pm$ 1.32 ^{b)*} (5)
Liver††	10.12 $\pm$ 0.83 (5)	5.97 $\pm$ 0.61 ^{b)} (5)	10.36 $\pm$ 1.16 (5)	6.39 $\pm$ 1.23 ^{b)} (5)
Brain††	2.88 $\pm$ 0.53 (4)	3.16 $\pm$ 0.40 (5)	2.81 $\pm$ 0.44 (5)	3.12 $\pm$ 0.21 (4)

† $\mu\text{g}/100\text{ ml}$; †† $\mu\text{g}/\text{g}$, fresh tissue.

Each figure is mean $\pm$ S.E. of number of samples given in parenthesis: a) $p < 0.001$, b) $p < 0.01$, c) $p < 0.05$ versus saline control; * $p < 0.05$ versus corresponding Pb-sodium acetate administered group as evaluated by the student's 't' test.

the levels of brain Pb, biogenic amines and the activities of renal enzymes irrespective of renal condition (Tables 2, 4 and 5).

Table 3. Effect of DMPS on Pb induced alterations in blood parameters in rats

	Pb-sodium acetate		Pb-uranyl acetate	
	saline	DMPS	saline	DMPS
δ -ALAD†	3.16±0.53 (5)	5.50±0.78 ^{b)} (5)	1.13±0.10 (5)	2.99±0.52 ^{b)} * (5)
ZPP††	6.86±2.59 (5)	3.74±0.84 ^{b)} (5)	12.10±1.91 (5)	6.72±0.80 ^{a)} * (5)
Hb†††	10.35±0.46 (5)	10.50±0.33 (5)	10.32±0.38 (5)	10.86±0.53 (6)
PCV††††	41.50±1.25 (5)	39.66±3.06 (6)	39.75±2.42 (5)	41.57±0.65 (6)

† μ mole δ -ALA/min/1-erythrocyte; †† μ g ZPP/g, Hb; ††† g/100 ml; †††† % volume. Each figure is mean±S.E. of number of samples given in parenthesis: ^{a)} $p < 0.001$, ^{b)} $p < 0.01$ versus saline control * $p < 0.05$, versus corresponding Pb-sodium acetate treated group as evaluated by the student's 't' test.

Table 4. Effect of DMPS on Pb induced alterations in brain biogenic amines in rats

	Pb-sodium acetate		Pb-uranyl acetate	
	saline	DMPS	saline	DMPS
DA†	0.35±0.03	0.35±0.04	0.33±0.02	0.34±0.02
NE†	0.27±0.01	0.31±0.02	0.26±0.02	0.26±0.03
5-HT†	0.41±0.03	0.46±0.03	0.50±0.05	0.44±0.01

Each figure is mean±S.E. of 5 samples.

† μ g/g, fresh tissue.

Table 5. Effect of DMPS on Pb induced alterations in renal enzymes in rats

	Pb-sodium acetate		Pb-uranyl acetate	
	saline	DMPS	saline	DMPS
LDH†	1.58±0.12	1.78±0.14	1.03±0.14	1.11±0.06**
GOT††	30.14±2.06	32.09±2.59	16.34±2.77	17.80±1.48**
ALP†††	0.83±0.17	0.71±0.02	0.44±0.02	0.42±0.01**

† μ mole NADH oxidized/min/mg protein; †† n mole of hydrazone formed/min/mg protein; ††† μ mole phenol liberated/min/mg protein. Each figure is mean±S.E. of 6 samples; ** $p < 0.01$ versus corresponding Pb-sodium acetate treated group as evaluated by students 't' test.

DISCUSSION

DMPS was effective in enhancing the urinary excretion of Pb, in lowering blood and tissue concentrations of Pb, reducing Pb induced urinary excretion of δ -ALA and in restoring the decreased activity of blood δ -ALA-D in rats which show its ability to form readily excretable complex with Pb *in vivo*. These effects were more marked in animals exposed to Pb-sodium acetate than in those exposed to Pb-uranyl acetate, showing comparatively poor elimination of Pb from the animals

with renal dysfunction. This may be explained by a) The blood supply in damaged kidneys would be poor and therefore, less chelating agent would reach the renal tissue to mobilize Pb into urine as urinary pathway is the main route of Pb elimination from the body. This is apparently true as blood levels of Pb decreased more markedly in rats with normal kidneys than in those with acutely damaged kidneys after cessation of Pb exposure before treatment with DMPS (Compare Tables 1 and 2); b) Due to kidney damage the renal clearance of Pb would be adversely affected and its retention would increase. This is supported by the fact that renal concentration of Pb was significantly higher in rats exposed to Pb-uranyl acetate than in those exposed to Pb-sodium acetate (Table 1) and c) The proximal tubular damage by uranyl acetate will lead to increased reabsorption of Pb during mobilization by DMPS resulting in lower elimination and higher retention of Pb in the renal tissue, which is indicated by comparatively less elimination of renal Pb in Pb-uranyl acetate exposed animals than in those exposed to Pb-sodium acetate (Table 2). However, no significant difference could be observed in the removal of hepatic-Pb by DMPS between rats with normal and damaged kidneys.¹⁹⁾

The results show DMPS to be an effective chelating agent for the treatment of Pb intoxication, irrespective of the renal function. However, supportive therapy for renal damage may be necessary to achieve optimum effect of DMPS in patients of Pb poisoning with renal dysfunction. As the increased brain level of Pb and altered levels of brain catecholamines remained unaffected on treatment with DMPS, the chelating agent has apparently failed to cross the blood-brain barrier or reach the neuronal site of lead accumulation.²⁰⁾

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