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Therapeutic potential of meso 2,3-dimercaptosuccinic acid or 2,3-dimercaptopropane 1-sulfonate in chronic arsenic intoxication in rats

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The therapeutic efficacy of two thiol chelators, meso 2,3-dimercaptosuccinic acid (DMSA) or 2,3-dimercaptopropane sulfonate (DMPS) in treating chronic arsenic intoxication was investigated in male rats. Both the chelators were effective in promoting urinary arsenic excretion and restoring arsenic induced inhibition of blood δ-aminolevulinic acid dehydratase activity and hepatic glutathione level. Elevation of urinary δ-aminolevulinic acid excretion and arsenic concentration in blood, liver and kidneys were reduced significantly by both the chelators. Histopathological lesions induced by arsenic were also effectively reduced by the above chelators. DMSA being more effective than DMPS. The results suggest DMSA and DMPS to be effective antidotes for treating chronic arsenic toxicity in experimental animals.

Keywords: arsenic, chronic toxicity, chelation, DMSA, DMPS, rat

Introduction

Toxicity of arsenic has long been of concern due to the frequent use of arsenicals as herbicides, insecticides, rodenticides, paint pigments, wood preservatives, and from the waste derived from the production of several metals and as a by-product of the uses of fossil fuel (Baxley *et al.* 1981). Environmental arsenic exposure has received attention primarily because of disease resulting from ingestion of water containing this element. Acute exposure of arsenic may produce immediate gastro-intestinal symptoms, sub acute sequela resulting in polyneuropathy (Goebel *et al.* 1990). Chronic effects may associate arsenic with degenerative, inflammatory and neoplastic changes of the skin, respiratory system, liver, haematopoietic, cardiovascular, nervous and reproductive system (Neiger & Osweiler 1990). For many years British Anti-Lewisite (BAL), commonly known as dimercaprol (2,3-dimercapto-1-propanol), has been used for the treatment of poisoning by compounds of arsenic, lead and mercury (Hammond & Bieliles 1986). BAL was originally developed to treat the effects of lewisite (dichloro (2-chlorovinyl) arsine), i.e. systemic poisoning and local skin vesication. In the former role, BAL suffers the disadvantages of a low safety ratio, unpleasant side effects and difficulties in systemic administration (Aposhian 1983, Graziano 1986, Sulzberger *et al.* 1946).

The chemically-related analogs of dimercaprol, meso 2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercaptopropane 1-sulfonate (DMPS) are more water soluble, orally active and markedly less toxic when compared with BAL (Aposhian 1983, Aposhian & Aposhian 1990). These chelating agents are found to be effective against poisoning by compounds of arsenic, lead, mercury, cadmium, cobalt, silver, etc. (Aposhian 1990). DMPS is used clinically in Russia as a standard chelating agent under the name of Unithiol (Klimova 1958). DMSA, also known as Succimer, has recently been approved by the USFDA for treating childhood lead poisoning. DMPS has been considered to have the same mechanism of metal excretion as DMSA (Planas-Bohne *et al.* 1980). However, there are few studies comparing these two antidotes against chronic arsenic poisoning. The present study concerns the therapeutic efficacy of DMSA and DMPS in the mobilization and distribution of arsenic following sodium arsenate administration.

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Materials and methods

Dibasic sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) was purchased from Sigma. Male albino rats (weighing approximately 120 g) from our establishment animal facility were used. The animals were freely supplied with pelleted diet (Lipton's India Ltd; metal contents of diet Cu 10.0, Zn 45.0, Fe 70.0, Mn 55.0, Co 5.0) and were housed in an air-conditioned facility. Animals were given arsenic as sodium arsenate (1 mg kg^{-1} dissolved in distilled water) orally 6 days a week for 3 weeks. Six animals received distilled water through the same route and served as controls. After 3 weeks of arsenic exposed the animals were divided equally into three groups of six rats each and treated three times daily, orally for 5 days as indicated:

Group I 4 ml kg^{-1}, normal saline
Group II 50 mg kg^{-1} DMSA
Group III 50 mg kg^{-1} DMPS

All the chelating agents were prepared daily and the pH adjusted to 6.4 with sodium bicarbonate. The animals were kept individually in metabolic cages and 24 h urine was collected for five consecutive days. The animals were sacrificed 48 h after the last administration. Kidney, liver and brain were removed, and blood was collected from the heart in heparinized tubes.

Biochemical assays

The activity of blood δ-aminolevulinic acid dehydratase (ALAD) was assayed according to the procedure of Berlin & Schaller (1974). The urinary excretion of δ-aminolevulinic acid (ALA) was measured using a dual ion exchange chromatographic procedure (Davis *et al.* 1968). Zinc protoporphyrin (ZPP) was estimated in a drop of blood using a hematofluorometer (Model ZPP; Aviv, Lakewood NJ) (Grandjean 1979). Hepatic glutathione contents were measured following the method of Ellman (1959). Activities of serum glutamic oxaloacetic (GOT) and glutamic pyruvic transaminase (GPT) were determined following the method of Reitman & Frankel (1957).

Metal estimation

Measurement of arsenic in blood, urine and kidney were carried out using an atomic absorption spectrophotometer (Spectra AA 30/40; Varian, Australia) fitted with a graphite furnace following wet acid digestion.

Histopathological observations

Liver and kidneys were removed, rinsed with normal saline and cut into small bits. Tissue bits were fixed in aqueous Bouins fluid and 3–4 μm thin sections were stained with haematoxylin & eosin. Histopathological changes were observed by light microscopy.

Results and discussion

Arsenic exposure produced significant inhibition of blood ALAD activity, and elevation of blood ZPP and urinary ALA excretion. Treatment with both DMSA and DMPS produced a significant recovery in blood ALAD activity and urinary ALA excretion. However, no change in blood ZPP following treatment with the chelators was noticed (Table 1).

Table 2 indicates the effects of DMSA or DMPS on some biochemical variables in arsenic-exposed rats. Significant depletion in hepatic glutathione contents and a marginal elevation of serum GOT and serum GPT activities were observed on arsenic administration for 3 weeks. Treatment with DMSA and DMPS elevated the arsenic induced inhibition of the hepatic GSH level. However, no noticeable effect on serum GOT or serum GPT following treatment with DMSA was observed. Figure 1 indicates that urinary arsenic excretion increased on exposure. Treatment with both DMSA and DMPS was effective in potentiating arsenic elimination (DMSA being more effective). The arsenic concentrations of blood, liver, kidney and brain following treatment with DMSA or DMPS are given in Table 3. DMSA was more effective than DMPS in reducing the blood, hepatic and renal arsenic concentrations.

Renal histopathological observation revealed damaged glomeruli which were infiltrated with blood cells. Mild edema was observed in the glomeruli at a few places

Table 1. Influence of DMSA/DMPS treatment on arsenic induced hematopoietic alterations in rats

	Blood ALAD (nmol min^{-1} ml^{-1} erythrocyte^{-1})	Blood ZPP (μg g^{-1} Hb)	Urine ALA (mg 100 ml^{-1})
Normal animals	5.79 ± 0.17	0.70 ± 0.12	0.062 ± 0.001
Arsenic Control	2.03 ± 0.36*	1.90 ± 0.19*	0.140 ± 0.006*
DMSA	4.00 ± 0.69[a]	2.20 ± 0.10	0.096 ± 0.005[b]
DMPS	4.74 ± 0.10[a]	1.90 ± 0.10	0.101 ± 0.001[b]

Values are mean ± SE; $N = 6$.
*$P < 0.001$ compared with normal animals; [a]$P < 0.001$, [b]$P < 0.01$ compared with arsenic-exposed controls as evaluated by Student's t-test.

Table 2. Influence of DMSA/DMPS treatment on some arsenic-induced biochemical alterations in hepatic and serum enzymes in rats

	Hepatic GSH	Serum GOT	Serum GPT
Normal animals	6.26 ± 0.23	3.76 ± 0.19	3.27 ± 0.10
Arsenic control	5.08 ± 0.29	4.09 ± 0.18	4.10 ± 0.24
DMSA	7.37 ± 0.66[b]	3.97 ± 0.33	4.47 ± 0.22
DMPS	8.10 ± 0.56[a]	4.34 ± 0.18	4.11 ± 0.20

Units: GSH, μg g^{-1}; serum GOT and GPT, μmol hydrazone formed min^{-1} mg $protein^{-1}$.
Values are mean ± SE, $N = 6$.
[a]$P < 0.001$, [b]$P < 0.05$ compared with arsenic-exposed controls as evaluated by Student's t-test.

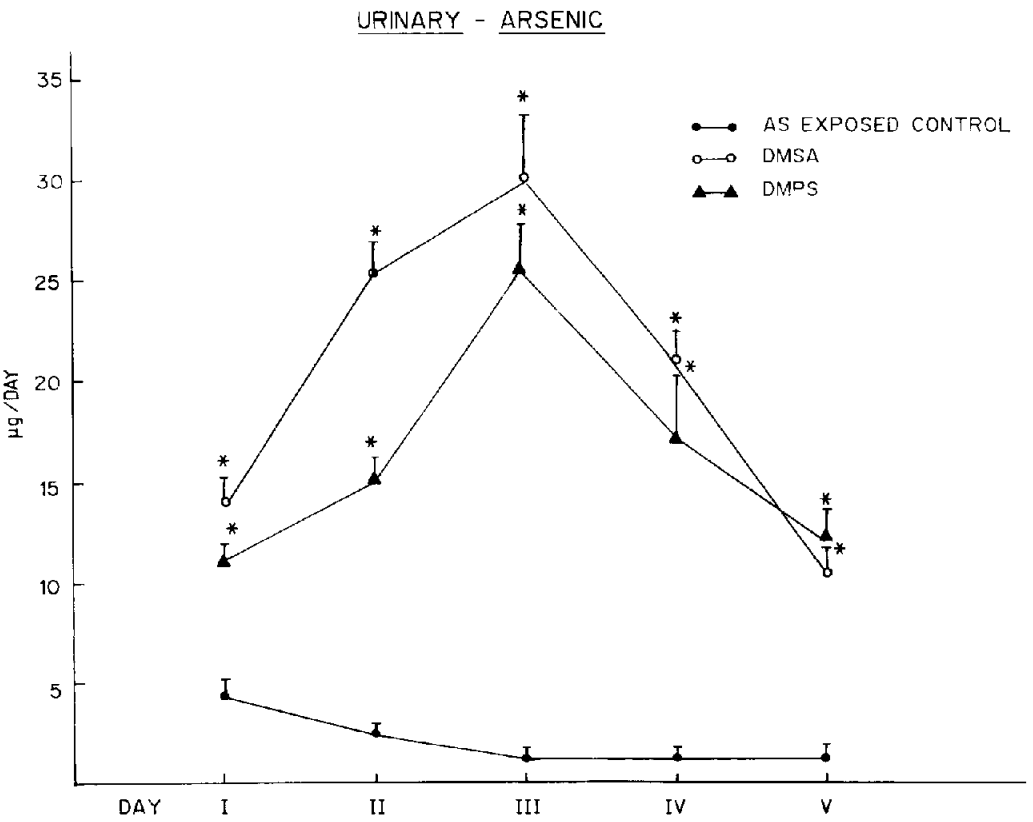

Figure 1. Influence of DMSA/DMPS administration on urinary arsenic excretion in arsenic poisoned rats. Each point is mean ± SE of five values. $^{*}P < 0.05$ compared with arsenic-exposed controls as evaluated by Student's *t*-test.

Table 3. Influence of DMSA/DMPS treatment on the arsenic concentration in poisoned rats

	Blood ($\mu g\ 100\ ml^{-1}$)	Liver ($\mu g\ g^{-1}$)	Kidney ($\mu g\ g^{-1}$)
Normal animal	0.66 ± 0.17	0.18 ± 0.09	0.08 ± 0.02
Arsenic control	20.34 ± 2.36*	8.42 ± 1.25*	6.49 ± 0.70*
DMSA	8.07 ± 0.98[a]	4.38 ± 0.63[a]	3.27 ± 0.31[a]
DMPS	14.47 ± 1.02[a]	6.08 ± 1.10[a]	4.59 ± 0.21[b]

Values are mean ± SE. $N = 6$.
$^{*}P < 0.001$ compared with normal animals. [a]$P < 0.001$, [b]$P < 0.05$ compared with arsenic-exposed controls as evaluated by Student's *t*-test.

following arsenic exposure. Nuclei of the epithelial lining of the proximal tubules were damaged (Figure 2b) as compared with normal histoarchitecture (Figure 2a). Histopathological lesions in hepatic parenchyma following arsenic exposure included damage in the portal triad area of the hepatic lobule, vascular congestion and mild edema at a few places (Figure 3b) when compared to control liver section (Figure 3a). Treatment with DMSA and DMPS provided partial recoveries in renal and hepatic histopathological lesions. However, mild to moderate renal tubular edema and mild damage in the epithelial lining of renal tubules were noticed on DMSA treatment. Nuclei with a nucleolus were indicative of regenerative changes following DMSA treatment (Figure 2c). Renal sections of DMPS treated animals showed necrotic nuclei of the epithelial lining of the tubules (Figure 2d). DMSA produced mild congestion in the portal triad and cord pattern was also maintained. Partial regenerative changes were observed in the hepatocytes (Figure 3c). On the other hand, treatment with DMPS induced moderate congestion in the central canal and decreased the nuclear density due to damaged hepatocytes nuclei (Figure 3d).

Chelation therapy is widely used in the management of metallic poisoning. The assay of agents that bind and/or mobilize metals can be based on a number of different measurable responses. The basis of one type of assay is the increased excretion of the metal by metal binding agents (Cantilena & Klaassen 1982). A second assay of agents that bind metals is the prevention of the lethal effects of the particular metals by the chelating agent (Aposhian *et al.* 1981, Hilmy 1991). In human and most of animals studied in experiments, the major route of arsenic excretion is through the kidney with only marginal elimination through feces (Vahter 1981). In our experiment, treatment with both DMSA and DMPS was effective in reducing the arsenic body burden and increasing urinary arsenic excretion (DMSA being more effective than DMPS). Histopathological lesion induced by arsenic also recovered more

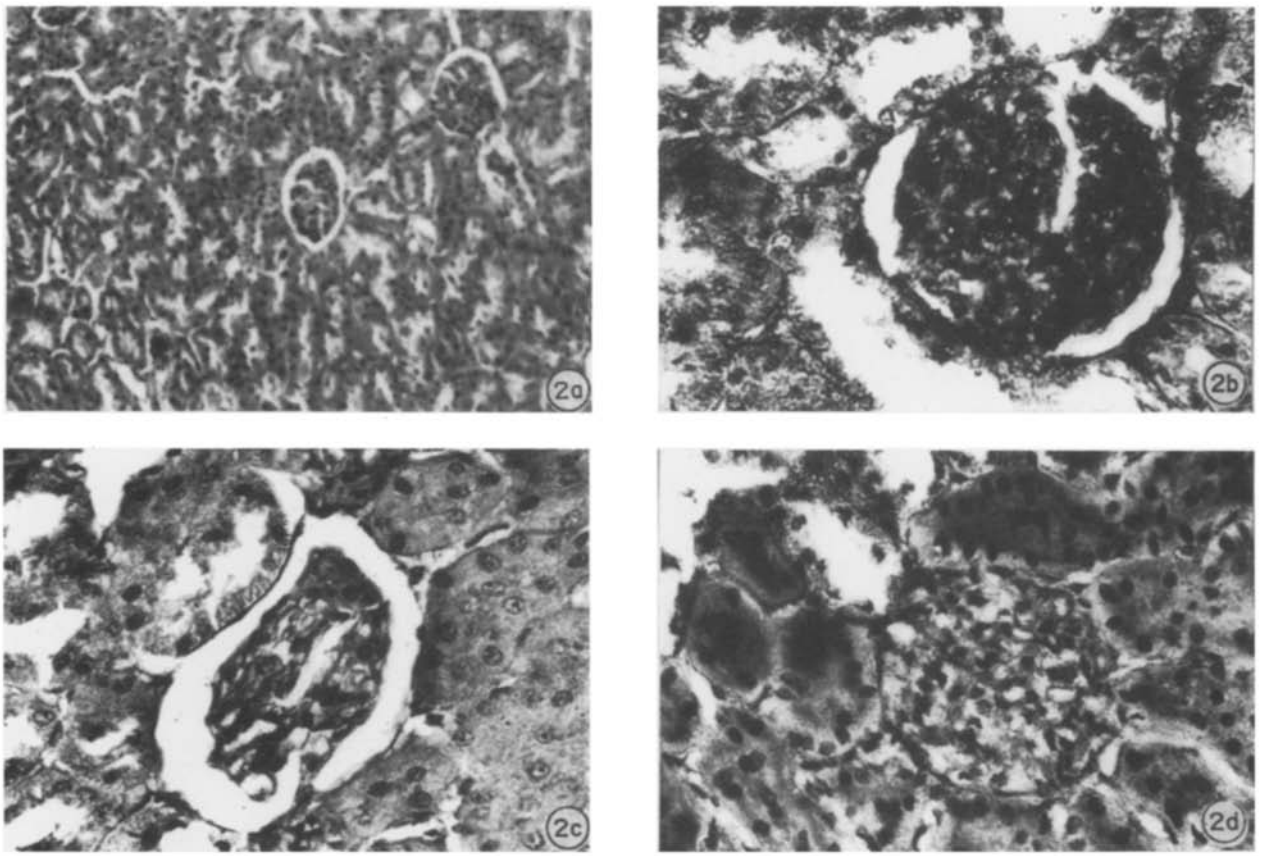

Figure 2. (**a**) Light photomicrograph of renal proximal tubules cells from a control rat showing normal features, ×80. (**b**) Following arsenic exposure, damaged glomerular and epithelial linings and decreased nuclear density were noticed, ×288. (**c**) Treatment with DMSA produced mild to moderate renal tubular edema, increased nuclear density and the nuclei contained a nucleolus, ×288. (**d**) Compared with DMSA, less marked recoveries were observed in renal tubules of DMPS-treated rats with necrotic nuclei in epithelium of the tubules, ×288.

prominently following DMSA administration than DMPS. BAL was primarily designed as an antidote against certain arsenical compounds, i.e. Lewisite. In Western Europe and the US it has remained the standard therapy for arsenic poisoning (Klaassan 1985), although other investigators have recommended DMPS (Luganski & Loboda 1960, Reichi *et al.* 1990). Aposhian *et al.* (1981) suggested that DMPS and DMSA were more effective than BAL in the treatment of Lewisite poisoning in rabbits. There appears to be little to choose between BAL and the other two water soluble chelating agents, DMSA and DMPS. However, it is essential to take in account the toxicity and physical properties of these chelating agents. It is clear that BAL is considerably more toxic than DMPS or DMSA (Aposhian 1983). In addition, a higher molar dose of DMSA or DMPS can be administered. The results of the experiments reported in this paper clearly show the beneficial effects of DMSA and DMPS in treating chronic arsenic toxicity. Comparing the beneficial role of the two chelators, it is clear that DMSA is more effective than DMPS. The tissue arsenic concentration reduced more rapidly under DMSA treatment than DMPS treatment. A significant elevation of arsenic-induced hepatic glutathione depletion is also a most noteworthy finding, and perhaps may indicate the possible mechanisms of protection by DMSA and DMPS. We have not included BAL in our study as a number of reports are available. However, BAL is approximately seven times more toxic than DMPS and 19 times more toxic than DMSA based on BAL LD_{50} (Zvirblis & Ellins 1976, Aposhian *et al.* 1981).

It can be concluded that DMSA and DMPS have significant therapeutic values in treating chronic arsenic intoxication in rats.

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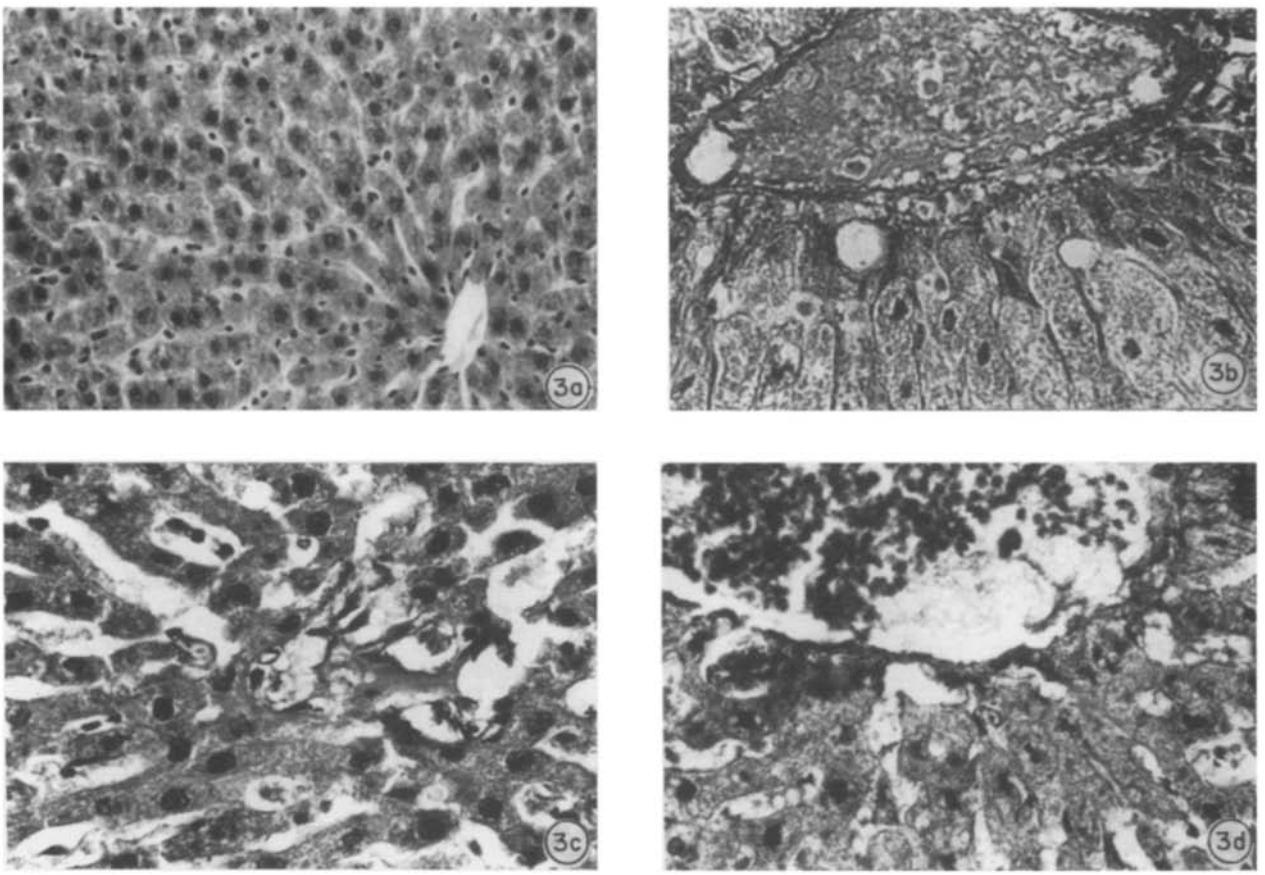

Figure 3. (**a**) Photomicrograph of rat liver from a normal control rat showing typical morphological architecture, ×128. (**b**) Arsenic exposure resulted in edema, congestion and nuclear degeneration, ×288. (**c**) Treatment with DMSA induced a characteristic deeply basophilic stained nuclei and increased nuclear density. ×288. (**d**) Congestion in central canal and decreased nuclear density were observed following treatment with DMPS indicating less marked therapeutic effects, ×288.

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