

## **Therapeutic Efficacy of a Few Diesters of Meso 2,3-Dimercaptosuccinic Acid during Sub-Chronic Arsenic Intoxication in Rats**

S.J.S. FLORA<sup>1</sup>, B.P. PANT<sup>2</sup>, Neelima TRIPATHI<sup>1</sup>, G.M. KANNAN<sup>1</sup> and D.K. JAISWAL<sup>2</sup>

<sup>1</sup>Division of Pharmacology and Toxicology and

<sup>2</sup>Division of Chemistry, Defence Research and Development Establishment

**Abstract: Therapeutic Efficacy of a Few Diesters of Meso 2,3-Dimercaptosuccinic Acid during Sub-Chronic Arsenic Intoxication in Rats: S.J.S. FLORA, et al. Division of Pharmacology and Toxicology, Defence Research and Development Establishment**—The therapeutic efficacy of four diesters derivatives of meso 2,3-dimercaptosuccinic acid (DMSA) was investigated in subchronic arsenic intoxication in rats. Dimethyl DMSA (DMDMSA), diethyl DMSA (DEDMSA), diisopropyl DMSA (DiPDMSA) and diisoamyl DMSA (DiADMSA) were the diesters of DMSA with methyl, ethyl, isopropyl and isoamyl alcohols and were administered for two 5 day courses of treatment in male rats pre-exposed to 100 ppm arsenic (III) for 8 weeks. The results show that treatment with these diesters was effective in decreasing blood and soft tissue arsenic contents but was only moderately effective in recovering biochemical indicators. The results suggest that these diesters could be effective arsenic chelators but may be inferior to DMSA in recovering biochemical/clinical indicators following sub-chronic arsenic exposure. (*J Occup Health 1997; 39: 119-123*)

**Key words:** Arsenic toxicity, Sub-chronic exposure, Biochemical alteration, Arsenic concentration, Chelation, Diesters, Rats

The toxicity of arsenic has long been of concern due to the frequent use of arsenicals as herbicides, insecticides, rodenticides, paint pigments and wood preservatives. Arsenic occurs in the wastage derived from the production of several metals and as a by-product of the use of fossil fuels<sup>1</sup>. Environmental arsenic exposures have drawn attention primarily because of diseases resulting from ingestion of water containing this element.

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*Correspondence to: S.J.S. Flora, Division of Pharmacology and Toxicology, Defence Research and Development Establishment, Jhansi Road, Gwalior-474 002, India*

British Anti Lewisite (BAL) has been used for the treatment of arsenic poisoning<sup>2</sup> but its use is handicapped by the limitation to intramuscular administration and its small therapeutic doses<sup>3,4</sup>. The dithiols, meso 2,3-dimercapto succinic acid (DMSA) and sodium 2,3-dimercaptopropane 1-sulfonate (DMPS) can be administered intravenously or orally. They have been found to be very effective in the treatment of heavy metal poisoning particularly lead, cadmium and arsenic. DMSA has been found to be as effective as Ca disodium EDTA in the treatment of occupational lead poisoning and as effective as DMPS in the treatment of mercury poisoning<sup>5,6</sup>. DMSA and DMPS have been reported to be effective in reducing the lethality of trivalent arsenicals in experimental animals<sup>7</sup>. Equimolar doses of DMSA and DMPS reduce the death rate of animals poisoned with arsenic more effectively than BAL<sup>8,9</sup>, and, because of their lower toxicity, DMPS and DMSA can be administered at much higher doses than BAL. The therapeutic effect of the dithiols is attributed to the ability of their vicinal thiol groups to react with trivalent arsenicals forming a saturated five membered heterocyclic ring. Following this, mobilization of arsenic from the body increases<sup>10,11</sup>. It has been suggested that active transport of DMPS into cells occurs via an anion carrier system<sup>12</sup>. There is very little to choose between DMSA and DMSP as far as their ability to chelate is concerned<sup>10,13,14</sup>, but the hydrophilic properties of DMSA do not allow the antidote to pass through the cell membranes and to capture intracellular arsenicals<sup>12</sup>. New dimercaptosuccinic acid (DMSA) analogues with more lipophilic properties were recently synthesized<sup>15</sup>. These analogues have been found to be effective in reducing the lethality of arsenic and eliminating it from various organs following acute arsenic exposures. Nevertheless, most of these data only indicate that these analogues are effective in reducing the body arsenic burden after acute exposure but their ability to recover altered biochemical/clinical indicators after sub-chronic exposure has not been studied. The present

study was therefore planned to assess whether i) these new derivatives of DMSA are effective antidotes even after chronic exposure, ii) these derivatives could provide simultaneous recoveries in altered biochemical indicators following sub-chronic exposure. The parent drug DMSA, a known highly effective arsenic chelating drug, was used in this study as a reference agent.

### Materials and Methods

**Chemicals:** Meso 2,3-dimercaptosuccinic acid and delta-aminolevulinic acid were purchased from Sigma Chemicals (St. Louis, MO, USA), Sodium arsenite was purchased from Merck (Germany). All other chemicals were of analytical grade. The diesters of DMSA were prepared as reported earlier<sup>16-18</sup>. The chemical structures of DMSA and the diesters are shown in Fig. 1. Meso DMSA was dissolved in 5% bicarbonate. Dimethyl DMSA (DMDMSA) and Diethyl DMSA (DEDMSA) were dissolved in ethanol and 5% bicarbonate, 1:5 because of poor water solubility. Diisooamyl DMSA (DiADMSA) and Diisopropyl DMSA (DiPDMSA) were dissolved in peanut oil.

**Animals and treatment:** Male Wistar albino rats (150 ± 10 g) from the Defence Research and Development Establishment colony were maintained on standard pellet diet (Lipton's India Ltd; metal contents of feed, in ppm dry weight; Cu 10.0, Mn 55, Co 5.0, Fe 70.0 and Zn 45.0) and water *ad libitum*. These rats had free access to 100 ppm sodium arsenite in drinking water. Five rats were given arsenic free drinking water *ad libitum* for 8 weeks. The arsenic exposed animals were randomly divided into six equal groups and treated daily for 5 days as follows;

- Group 1 - distilled water (4 ml/kg), orally through gastric gavage (control)
- Group 2 - saline (1.0 mmol/kg), orally
- Group 3 - DMDMSA (1.0 mmol/kg), orally
- Group 4 - DEDMSA (1.0 mmol/kg), orally
- Group 5 - DiPDMSA (1.0 mmol/kg), orally
- Group 6 - DiADMSA (1.0 mmol/kg), orally
- Group 7 - DMSA (1.0 mmol/kg), orally.

After the first 5 day course of chelation, the animals were left without any treatment for 5 days. Thereafter, the animals were given the second 5 day course of treatment with the chelating agents. The doses and the route of administration for the chelating agents were selected after careful review of literature<sup>5,6,10</sup>. After the final dose of chelators, 24 hr urine samples were collected for a certain biochemical estimations and blood was collected in heparinized tubes from the retro-orbital plexus of the rats. All the animals were decapitated and the liver, kidneys and brain were removed.

**Biochemical Assays:** Standard procedures were used to measure the activity of blood  $\delta$ -aminolevulinic acid dehydratase (ALAD)<sup>19</sup>, blood zinc protoporphyrin (ZPP)<sup>20</sup> and urinary  $\delta$ -aminolevulinic acid (ALA)<sup>21</sup>. We have mentioned in a few earlier reports from our laboratory that

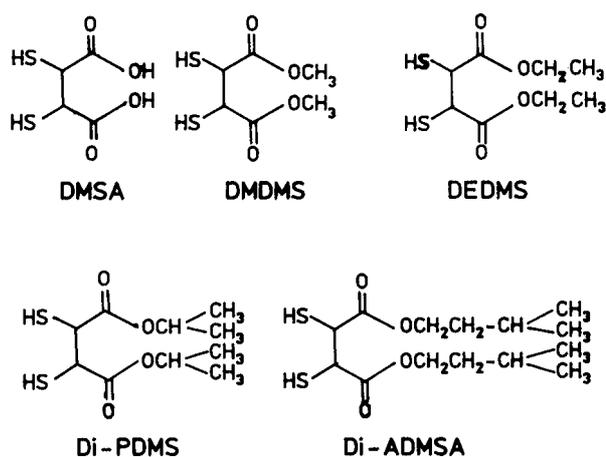


Fig. 1. Structures of the meso-2,3, dimercaptosuccinic acid and esters.

these biochemical indicators are sensitive to any increase in the body arsenic burden<sup>10</sup>.

The activities of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined following the procedure of Reitman and Frankel<sup>22</sup>. The plasma alkaline phosphatase (ALP)  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) and lactate dehydrogenase (LDH) activities were determined with commercially available diagnostic kits from Ranbaxy India Ltd. The activities of ALP,  $\gamma$ -GT and LDH were determined by kinetic methods taking p-nitrophenyl phosphate,  $\gamma$ -glutamyl p-nitroanilide and NADH respectively, as the substrate. The activities were measured at 405 nm for ALP and  $\gamma$ -GT and 340 nm for LDH with a Milton Roy Spectronic 1201 Spectrophotometer. Reduced glutathione (GSH) contents were determined following the procedure of Ellman *et al.*<sup>23</sup>.

**Metal estimation:** Arsenic concentrations in blood, liver, kidney and brain were determined with an atomic absorption spectrophotometer (AAS) following wet acid digestion<sup>24</sup>.

### Results

Sub-chronic exposure to arsenic produced a significant inhibition in blood ALAD activity accompanied by an increase in the excretion of urinary ALA (Table 1). There was also a decrease in blood haemoglobin content and an increase in blood ZPP. Two 5-day courses of DMSA or diesters produced only marginal recovery in the above altered variables with DMSA, diethyl and diisopropyl esters being almost equally effective in increasing inhibited blood ALAD activity, although the increase in blood ALAD activity caused by diethyl DMSA was not statistically significant. No influence of DMSA or diesters on other blood indicators was observed except for a significant recovery in urinary ALA excretion following DMSA administration (Table 1).

The effects of arsenic exposure and subsequent treatment

**Table 1.** Efficacy of various DMSA derivatives on arsenic induced alterations on some haematopoietic biochemical indicators in rats

	Blood ALAD n mol/min/ml RBC	ZPP µg/g	Hb g/100 ml	Urine ALA mg/100 ml
Control	6.13 ± 0.12	0.44 ± 0.05	13.49 ± 0.55	0.45 ± 0.03
As (control)	2.89 ± 0.26 <sup>a</sup>	1.62 ± 0.14 <sup>a</sup>	11.63 ± 0.16 <sup>a</sup>	2.20 ± 0.40 <sup>a</sup>
Dimethyl DMSA	3.06 ± 0.11	1.44 ± 0.08	11.41 ± 0.78	2.20 ± 0.40
Diethyl DMSA	3.61 ± 0.24	1.42 ± 0.15	11.27 ± 0.18	1.80 ± 0.10
Diisopropyl DMSA	3.94 ± 0.15*	1.54 ± 0.12	11.15 ± 0.59	1.50 ± 0.09
Diisoamyl DMSA	3.02 ± 0.19	1.44 ± 0.06	11.65 ± 0.22	2.20 ± 0.03
DMSA	3.84 ± 0.15*	1.50 ± 0.11	11.70 ± 0.74	1.10 ± 0.06*

Values are the mean ± SE; n=5. <sup>a</sup> p<0.05 compared to normal animals; \* p<0.05 compared to arsenic exposed (Control) rats as evaluated by the Student's t-test.

**Table 2.** Efficacy of various DMSA derivatives on arsenic induced alterations on certain serum and plasma enzymes activities in rats

	Serum GOT (µ mol/min/mg hydrazone formed/mg protein)	Serum GPT	Plasma γ-GT	Plasma LDH (IU/L)	Plasma ALP (IU/L)
Control	4.08 ± 0.09	3.92 ± 0.09	11.82 ± 0.60	105.2 ± 1.26	114.20 ± 2.17
As (Control)	5.10 ± 0.15 <sup>a</sup>	5.18 ± 0.37 <sup>a</sup>	20.11 ± 0.44 <sup>a</sup>	112.6 ± 3.54 <sup>a</sup>	149.60 ± 6.76 <sup>a</sup>
Dimethyl DMSA	4.44 ± 0.35	5.02 ± 0.26	22.17 ± 1.18	114.4 ± 5.41	156.20 ± 6.68
Diethyl DMSA	4.64 ± 0.11	5.04 ± 0.56	21.04 ± 1.68	116.6 ± 6.41	137.06 ± 10.98
Diisopropyl DMSA	4.11 ± 0.76	4.67 ± 0.23	19.81 ± 1.03	114.8 ± 4.45	136.80 ± 9.75
Diisoamyl DMSA	4.21 ± 0.46	4.86 ± 0.17	20.95 ± 1.85	118.2 ± 6.12	141.04 ± 6.10
DMSA	5.40 ± 0.21	5.23 ± 0.24	21.36 ± 1.98	109.8 ± 4.17	135.60 ± 9.14

Values are the mean ± SE; n=5. <sup>a</sup> p<0.05 compared to normal control rats as evaluated by the Student's t-test.

**Table 3.** Efficacy of DMSA or DMSA analogues on arsenic concentration in blood and organs in rats

	Blood (µg/100 ml)	Liver (µg/g)	Kidney (µg/g)	Brain (µg/g)
Control	0.11 ± 0.11	0.11 ± 0.01	0.16 ± 0.01	0.06 ± 0.01
As (Control)	14.80 ± 1.54 <sup>a</sup>	5.69 ± 0.46 <sup>a</sup>	4.66 ± 0.50 <sup>a</sup>	0.18 ± 0.03 <sup>a</sup>
Dimethyl	9.03 ± 1.02*	3.48 ± 0.25*	3.48 ± 0.69*	0.14 ± 0.02
Diethyl	6.94 ± 0.87*	3.21 ± 0.24*	2.06 ± 0.12*	0.14 ± 0.02
Diisopropyl	4.01 ± 0.45*	1.84 ± 0.11*	1.23 ± 0.14*	0.11 ± 0.02*
Diisoamyl	6.95 ± 1.24*	2.77 ± 0.22*	2.01 ± 0.42*	0.13 ± 0.01*
DMSA	3.05 ± 0.13*	1.43 ± 0.14*	0.85 ± 0.30*	0.15 ± 0.02

Values are the mean ± SE; n=5. <sup>a</sup> p<0.05 compared to normal animals, \* p<0.05 compared to arsenic (control) exposed rats as evaluated by the Student's t-test.

with DMSA-dieters on serum transaminase activities are shown in Table 2. The data show that arsenic exposure produced a significant increase in serum GOT and GPT activities indicating hepatotoxicity of arsenic. None of the diesters could produce a significant recoveries in the increased transaminase activities though there was apparently a marginal decrease noticed in the activities of these enzymes activities. The activities were marginally higher in DMSA treated rats than in arsenic exposed control

rats. This may be due to the effect of DMSA in addition to arsenic induced hepatotoxicity (Table 2).

Plasma LDH, ALP and γ-GT activities increased significantly on arsenic exposure (Table 2). None of the chelating agents was able to effectively reduce these high levels. Unlike the biochemical data, the effects of DMSA and its diesters on the arsenic concentration in blood and soft tissues presented an interesting picture (Table 3). All four diesters were effective in reducing the arsenic

concentration in blood and other organs, DiPDMSA being the most effective. But the effectiveness of these diesters was less than DMSA administered alone. All four diesters and DMSA were almost equally effective in lowering the brain arsenic level (Table 3).

### Discussion

Effective chelation therapy for intoxication by certain heavy metals depends on whether the chelating agents are able to reach the intracellular site where the heavy metal is firmly bound. Several compounds which have been found to be effective in chelating intra-cellularly bound metals include 2,3-dimercaptopropanol (BAL), dithiocarbamates and several esters of meso DMSA<sup>16,25</sup>. Dimethyl ester of meso DMSA has been shown to increase cadmium excretion, which indicates that this compound enters the cells<sup>26,27</sup>. All the three thiol compounds, BAL, DMSA and DMPS, have been reported to reduce the lethality of As(III) in experimental animals<sup>5</sup>. It has recently been proved, however, that DMPS and DMSA which effectively reduce the death rate of animals poisoned with arsenic (III) are better chelating drugs for arsenic toxicity than BAL because their toxicity is lower than that of BAL and they can be administered at much higher doses than BAL<sup>14</sup>. Though these dithiols react with arsenic, they cannot pass through the cell membranes nor capture intracellular arsenic because they are hydrophilic. Analogues of DMSA which have lipophilic properties have therefore been synthesized, but only a few of them have been found to be effective<sup>15,18</sup>. It was presumed that an antidote which is able to capture the arsenic both intra- and extra-cellularly might provide a greater therapeutic potential than a chelating agent which has an extra-cellular distribution property. Diisopropyl DMSA is the diester with the highest lipophilic character followed by diethyl and dimethyl. The reason for the less pronounced therapeutic efficacy of these diesters compared with DMSA could be that they might not get separated easily in the GI tract compared with DMSA which is readily absorbed. Kreppel *et al.*<sup>15</sup> also suggested that since these diesters are more lipophilic than DMSA, they might cross cell membrane and thus may be able to effectively remove brain arsenic. We did not, however, observe a significant difference between the brain arsenic contents of DMSA administered rats and the rats treated with diesters. Furthermore, the said decrease in the brain arsenic concentration following the administration of DMSA or diesters was without the redistribution of arsenic into any other major organ. The clinical biochemical indicators also responded less favorably to the treatment with DMSA and the diesters. There was on the other hand a marginal increase in S-GOT and S-GPT activities following DMSA administration in arsenic pre-exposed rats. These observations are in agreement with a few earlier reports indicating mild hepatotoxicity as one of the possible side effects of treatment with DMSA<sup>26-29</sup>. The results therefore

suggest that although these diesters are useful arsenic antidotes, they are certainly not superior to DMSA either in providing clinical recovery or decreasing the arsenic body burden.

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