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Therapeutic Efficacy of Dimercaptosuccinic Acid and Thiamine/Ascorbic Acid on Lead Intoxication in Rats

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Chelation therapy is a form of treatment aimed at removing a metal ion from a molecular site at which it is producing a "biochemical lesion". Chelation therapy should be instituted in symptomatic patients or in patients with a high blood lead concentration. The following requirement have been proposed for an ideal chelating agent (1) water solubility (2) resistance to metabolic degradation (3) ability to penetrate to metal storage sites (4) ready excretion by the kidney (5) ability to retain chelating ability at the pH of body fluid and (6) the property of forming metal complexes that are less toxic than the free metal ion. Another important property is greater affinity for the metal atom that of endogenous ligands. The large number of available ligands within the body makes the task of a chelating agent a formidable one (Levine 1975). The current treatment of lead poisoning is limited to a few chelating agents such as EDTA, BAL and D-penicillamine (Chisolm 1968). Their medical use is somewhat restricted because of toxic side-effects. Thus, the search for better therapeutic agents for treatment of lead poisoning is essential. BAL is a painful intramuscular injection and can cause nausea, vomiting and severe headache. Calcium disodium EDTA is also painful when given intramuscularly and is known for its nephrotoxicity. Pencillamine is not as effective as BAL or $CaNa_2EDTA$ but is administered orally. It can cause reactions resembling penicillin sensitivity, it is potentially nephrotoxic and cause neutropenia (Piomelli et al. 1984). 2,3-dimercaptosuccinic acid (DMSA) is relatively new water soluble chelating agent, structurally similar to BAL. It is currently receiving attention in the treatment of heavy metal poisoning (Aposhian 1983). The toxicity of DMSA

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is quite low. Its intraperitoneal LD50 in mice was 2.73 gm/kg; when it was given intravenously to rabbits and human being, recovery from urinary excretion was about 40% in half an hour and 80% in 4 hours (Shijun et al. 1988).

Thiamine, folic acid, pyridoxine and ascorbic acid either individually or in combination have been proven to be effective in reducing the toxic manifestations of lead and in enhancing the antidotal efficacy of $CaNa_2EDTA$ (Flora and Tandon 1986; Flora et al. 1986; Tandon et al. 1987). In a recent report from our laboratory, it was observed that given combination of thiamine and ascorbic acid with thiol chelators improved the ability of the animals to excrete lead thereby reducing body lead burden (Dhawan et al. 1988). In view of the beneficial effect of these two vitamin, it was considered of interest to evaluate their potential to modify the prophylactic action of DMS in lead intoxication in rat after repeated administration.

MATERIALS AND METHODS

Eighteen male albino rats (170±10 g) from Industrial Toxicology Research Centre colony were maintained on standard pellet diet (Hindustan Lever Ltd, India) ad libitum and 10 mg/kg lead as lead acetate, orally (4 ml/kg) for eight weeks. Six animals received unleaded water and served as control group. After completion of exposure period the lead treated animals were divided equally into four groups of six rats each and treated as follows.

Group I - Normal saline, 4 ml/kg, i.p.
Group II - DMSA, 0.1 m mole/kg, gastric gavage, once daily, for 5 days.
Group III - DMSA as in group II + Thiamine 10 mg/kg, intraperitoneally, once, daily.
Group IV - DMSA as in group II + Ascorbic Acid, 1% in drinking water (average intake 10-12 ml/rat/day).

DMSA solution was prepared by neutralization with $NaHCO_3$ immediately before use. The animals were kept in metabolic cages (1/cage) for collection of 24 hr urine for 5 consecutive days during the treatment. On day 7, blood was collected from the tail of each animal, in heparinized tubes.

For the next 5 days, animals were kept in separate cages without chelating agent treatment. However, supplementation of thiamine and ascorbic acid continued in group III and IV respectively during the above period. On day 13, animals were again kept in metabolic cages for the collection of 24 hr urine. On the following day blood from animals of each group was collected in heparinized vials and animals were given the same treatment as on the first 5 days. Urine was collected again for 5 consecutive days and the animals were sacrificed on day 21 by decapitation. Blood, liver, kidney and brain

collected. Standard procedures were employed to determine blood δ-aminolevulnic acid dehydratase (ALAD)(Berlin and Schallar 1974) -zinc protoporphyrin (ZPP) (Grandjean 1979) urine- δ-aminolevulinic acid (ALA), (Davis et al. 1968), -Pb (Kopito and Schwachmen 1967), and blood and tissue Pb (Yeager et al. 1971).

RESULTS AND DISCUSSION

The urinary excretion of lead in lead injected rats decreased gradually when the lead treatment was discontinued. As expected the urinary lead excretion increased in all other groups immediately after treatment with DMSA or DMSA + vitamin B_1 or DMSA + vitamin C. The efficacy of DMSA was confined mainly to the first two days of treatment, and the excretion of lead diminished on subsequent days of treatment. Combined therapy with DMSA + vitamin C excreted more lead than either DMSA alone or DMSA + vitamin B_1, particularly on first two days (Figure 1).

Estimation of blood ALAD activity, ZPP level and urinary ALA excretion, biological indicators of lead poisoning, are shown in Table 1. All three forms of treatment showed a reduction in ALA excretion, blood ZPP level and ALAD inhibition more prominently at 21 days. Treatment with DMSA + vitamin C was most effective than either of the two treatment at all interval.

Lead contents in blood and soft tissues are shown in Table 2. DMSA when given alone or in combination was effective in decreasing the hepatic, renal and blood lead levels. Brain lead, was significantly decreased by DMSA + vitamin C treatment.

DMSA is a water soluble chemical, analog of dimercaprol (Aposhian 1983). It is effective when given as an antidote for arsenic (Lenz 1981), lead (Friedheim et al.1978, 1981) and mercury (Friedheim and Corvi 1975) in human and experimental animals. The major route of excretion is via kidney (Aposhian 1983). DMSA in the treatment of lead poisoning in humans has been described by Wang et al. (1965). It has shown to be effective, safe and convenient. It has two adjacent-SH groups as potential lead complexing sites.

Deficiency of thiamine (Tokarski and Reio 1978) and alteration in ascorbic acid metabolism (Rudrapal et al. 1975) have shown to be associated with lead intoxication. Supplementation of these vitamins during chelation therapy in lead intoxication might be beneficial in alleviating the vitamin deficiency caused by lead and acting as complimentary agent (Flora et al. 1986). Supplementation of vitamin during chelation therapy could also change the cell permeability, facilitating removal of the intracellular bound metal from organs like brain also. Thiamine (Flora and Tandon 1986) and ascorbic acid (Papaioannou et al. 1978) has been shown to have protective effects against lead

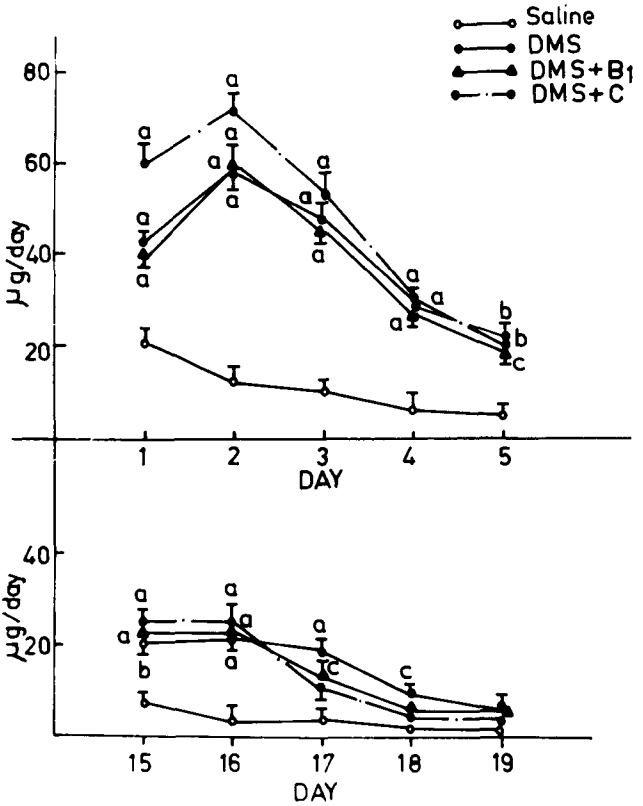

Figure 1. Effect of DMSA, DMSA + Vitamin B_1 or DMSA + Vitamin C on the urinary excretion of lead in lead administered rats. $^{a}p < 001$; $^{b}p < 0.01$, $^{c}p < 0.05$ compared to lead treated group as evaluated by the student's 't' test.

intoxication and to enhance the efficacy of $CaNa_2EDTA$ to counteract lead toxicity (Tandon et al. 1987). In the present study supplementation of ascorbic acid during DMSA therapy was more effective than either DMSA alone or DMSA + vitamin B_1 therapy. Supplementation of thiamine was not able to influence the efficacy of DMSA which are in agreement with the observation of Dhawan et al. (1988) that thiamine did not cause any major reduction in body lead burden.

The present results demonstrate DMSA to be an effective antidote of lead intoxication and combination of DMSA and ascorbic acid after repeated treatment further improved upon the performance in enhancing urinary lead excretion and reducing the body lead burden.

Table 1. Therapeutic efficacy of DMSA and thiamine/ascorbic acid after repeated administration on blood and urine parameters in lead intoxicated rats

	Blood ALAD* (n mol ALA/min/ml erythrocyte)			Blood ZPP** (µg ZPP/g hemoglobin)			Urinary ALA§ (mg/100 ml)		
	7	14	21	7	14	21	7	14	21
Control		12.2±0.5			1.05±0.32			0.08±0.01	
Lead		2.8±0.3[a]			4.40±0.13[a]			0.40±0.02[a]	
DMSA	3.3±0.2	3.7±0.2	4.3±0.4[z]	3.8±0.1[y]	3.8±0.2[z]	3.2±0.1[x]	0.32±0.01[y]	0.30±0.03[z]	0.18±0.02[x]
DMSA+Vit.B_1	3.0±0.2	2.7±0.2	5.2±0.4[y]	4.1±0.1	4.3±0.1	2.9±0.1[x]	0.30±0.04	0.23±0.01[x]	0.14±0.01[x]
DMSA+Vit.C	3.7±0.2[z]	4.5±0.2[y]	4.3±0.3[y]	3.2±0.1[x]	2.9±0.1[x]	1.7±0.1[x]	0.21±0.07[z]	0.14±0.08[z]	0.22±0.02[x]

* δ-aminolevulinic acid dehydratase; **Zinc protoporphyrin; § δ-aminolevulinic acid; each figure is mean ± SE, N=6, [a]$p < 0.001$ compared to normal control; [x]$p < 0.001$, [y]$p < 0.01$, [z]$p < 0.05$ compared to lead treated animals as evaluated by the Student's 't' test.

Table 2. Therapeutic efficacy of DMSA and thiamine/ascorbic acid after repeated administration on blood and tissue lead concentration in rats

	Blood (µg/100 ml)	Liver (µg/g)	Kidney (µg/g)	Brain (µg/g)
Control	7.18±0.82	0.75±0.07	0.97±0.05	0.04±0.01
Lead	69.17±2.44[a]	3.48±0.93[a]	7.48±0.65[a]	0.35±0.01[a]
DMSA	24.74±1.80[x]	1.10±0.08[x]	2.13±0.20[x]	0.33±0.01
DMSA+Vit.B_1	26.07±1.99[x]	1.32±0.13[x]	2.07±0.31[x]	0.30±0.01
DMSA+Vit.C	13.72±2.02[x]	0.95±0.04[x]	1.46±0.18[x]	0.25±0.01[x]

Each figure is mean ± SE; N=6, [a]$p < 0.001$ compared to normal control; [x]$p < 0.001$ compared to lead treated group as evaluated by the Student's 't' test.

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