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# Chelation in Metal Intoxication XXI: Chelation in Lead Intoxication During Vitamin B Complex Deficiency

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The exposure to lead decreases the concentration of riboflavin (Sessa et al. 1957) and thiamine (Tokarski and Reio 1978) in different tissues and nicotinic acid (Caccuri and Cesaro 1942) in blood and urine of experimental animals. The Vitamin B - complex deficiency increases the vulnerability to neuro- and systemic toxicity of Pb in young rats (Tandon et al. 1984). Conversely, the supplementation of Vitamin B - complex decreases the body uptake of Pb and reduces Pb induced biochemical alterations (Flora et al. 1984). Thus, the nutritional status of vitamins like that of protein or minerals seems to influence the etiology of Pb toxicity and may be expected to affect the response towards Pb chelators. 2,3 dimercaptosuccinic acid (DMSA) (Tandon et al. 1981) and N - (2 - hydroxyethyl) ethylenediamine triacetic acid (HEDTA) (Tandon et al. 1983) have been found to be effective antidotes to Pb intoxication.

In the present study, these selective metal chelating agents were compared for their ability to reduce the body burden of Pb and restore the altered biochemical parameters in young developing Pb intoxicated rats maintained on normal or vitamin B - complex deficient diet. The investigation was aimed to suggest suitable prophylaxis of Pb poisoning prevalent among children who may also be suffering from vitamin deficiency in developing and poor countries.

## MATERIALS AND METHODS

Forty-eight male albino rats (70±10g) of ITRC colony were used. Half of the animals were fed a normal synthetic diet and the remaining half were maintained on vitamin B - complex deficient diet throughout the experiment. The diets were prepared as per American Institute of Nutrition standards for nutritional studies (Bieri et al. 1977). Eighteen animals in each group

were administered 10 mg/kg, Pb as Pb $(OCOCH_3)_2$ $3H_20$ dissolved in distilled water, through gastric tube, 6 days a week for 6 weeks; the remaining six animals in each group received no treatment. The Pb exposed animals in each group were sub-divided equally into 3 groups. The animals of groups I and II were injected 0.3 m mole/kg, DMSA or HEDTA dissolved in normal saline (4 ml/kg), intraperitoneally, daily for 4 days and those of group III received an equal volume of saline alone. The pH of the injecting solution was adjusted to neutral using $NaHCO_3$.

The animals were kept in metabolic cages (1/cage) for the collection of 24 hr. urine samples, during 4 days of treatment. All the animals were killed by decapitation, 24 hr.after the last injection of chelating agent and blood, liver, kidney and brain were collected. Standard procedures were used to determine the activity of blood delta-aminolevulinic acid dehydratase (ALA-D) (Berlin and Schaller 1974), -zinc protoporphyrin (ZPP) (Grandjean 1979) and - haemoglobin contents (Clegg and King 1942); the urinary excretion of delta-aminolevulinic acid (ALA) (Davis et al.1968) and -Pb (Kopito and Schwachman 1967); the levels of brain dopamine (DA), -nor-epinephrine (NE) and -5-hydroxytryptamine (5-HT) (Sadavongvivad 1970). Lead was estimated in blood and tissues following nitric acid digestion, using atomic absorption spectrometer (Perkin-Elmer 5000) (Yeager et al. 1971).

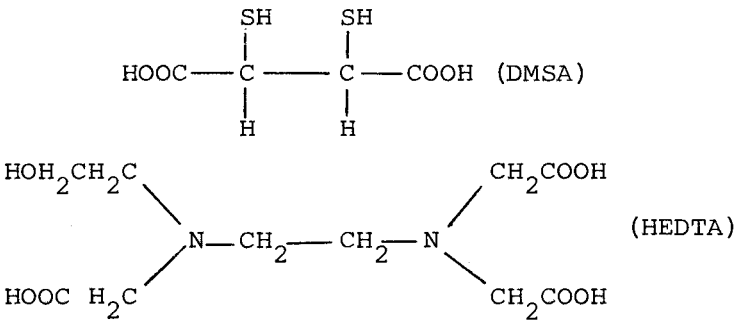


Figure 1. Structure of DMSA and HEDTA

RESULTS AND DISCUSSION

The urinary excretion of Pb increased and the Pb induced excretion of ALA decreased, more significantly on treatment with DMSA than with HEDTA. These effects were significantly less marked in animals fed vitamin deficient diet than in those fed normal diet (Figures 2 and 3).

The exposure to Pb inhibited the activity of blood ALA-D and increased blood ZPP more markedly in rats maintained on vitamin deficient diet than in those fed normal diet. The treatment with DMSA restored these alterations more effectively than with HEDTA. The effects of the chelators were generally more prominent in rats fed normal diet. The vitamin deficiency seems to decrease blood-Hb in Pb exposed animals which remained unaffected by chelation (Table 1). The administration of Pb for six weeks slightly decreased the levels of brain DA and 5-HT and increased the level of brain NE. None of the chelators could restore the altered levels of brain biogenic amines (not tabulated). The exposure to Pb caused more increase in blood, kidney, liver and brain Pb concentration in animals fed vitamin deficient diet than in those fed normal diet. The increased blood, kidney and liver Pb levels decreased more effectively on treatment with

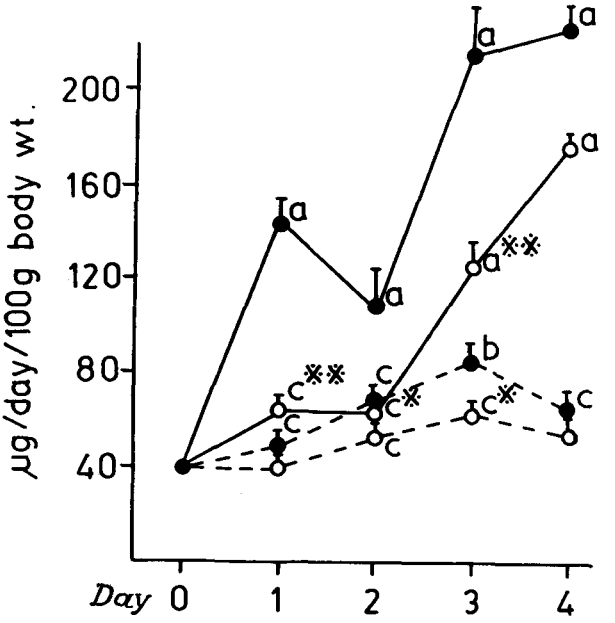


Figure 2. Urinary excretion of Pb upon treatment with DMSA (●—●) or HEDTA (●--●) in rats fed normal diet and DMSA (○—○) or HEDTA (○--○) in rats fed vitamin-B complex deficient diet. Each point is mean ± SE of 5 animals; $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ versus pre-treatment excretion; $^{**}p<0.01$, $^{*}p<0.05$ versus normal diet group at corresponding day, as evaluated by the Student's 't' test.

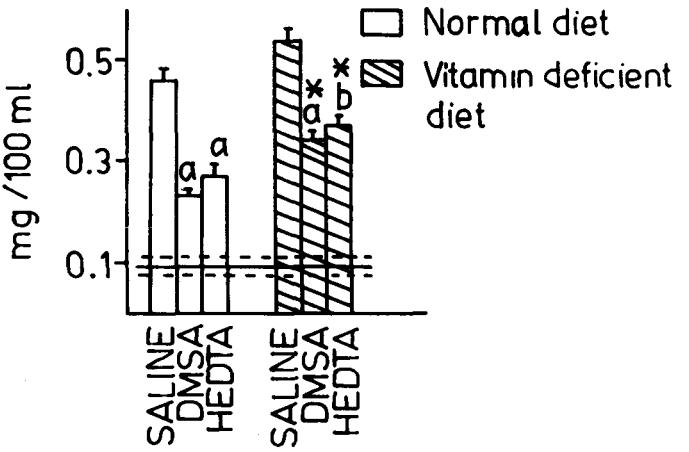


Figure 3. Urinary excretion of ALA 4 days after treatment with chelators in Pb poisoned rats fed normal diet or vitamin deficient diet. Each bar represents mean ± SE of 5 animals; $^{a}p<0.001$, $^{b}p<0.01$ versus saline group; $^{*}p<0.05$ versus normal diet group, as evaluated by the Student's 't' test. The horizontal line represents excretion in unexposed control group.

DMSA than with HEDTA. The Pb mobilization by the chelators particularly DMSA was significantly more marked in animals fed normal diet than in those fed vitamin deficient diet. The enhanced brain Pb level, however, remained unaffected by the chelation therapy (Table 2).

The exposure to Pb caused more marked biochemical alterations and increase in the Pb body burden in the developing rats fed vitamin deficient diet than in those fed normal diet. It was, therefore, of interest to investigate the response to chelation therapy of Pb poisoned animals maintained on two different diets. The ameliorative effects of DMSA and HEDTA were less marked in animals fed vitamin deficient diet as compared to those fed normal diet (Figures 2 and 3, Tables 1 and 2). This might be due to the more pronounced effects of Pb or the less elimination of Pb in vitamin deficient animals. Vitamin C has been shown to possess similar Pb chelating properties as equimolar amounts of ethylenediamine tetraacetic acid (EDTA) and their combination was more effective than either of them (Goyer and Cherian 1979) and thiamine (Vitamin $B_1$) has been found to potentiate the Pb antidotal properties of EDTA (Flora et al. 1986). The concomitant supplementation of vitamin B - complex with the administration of Pb reduced the accumulation of Pb in blood and vital

Table 1. Effect of chelators on lead induced alterations in blood parameters in rats fed normal or vitamin B - complex deficient diet.

| | Unexposed control | Normal diet Pb exposed | | | Unexposed control | Vitamin deficient diet Pb exposed | | |
|---|---|---|---|---|---|---|---|---|
| | | Saline | DMSA | HEDTA | | Saline | DMSA | HEDTA |
| ALA-D[+] | 4.8±0.21 | 2.6±0.26[x] | 4.1±0.24[a] | 2.9±0.41 | 4.4±0.26 | 2.0±0.20[x] | 3.2±0.41[b,*] | 2.4±0.16 |
| ZPP[++] | 2.9±0.42 | 8.9±0.46[x] | 5.1±0.53[b] | 5.6±0.36[b] | 3.8±0.41 | 11.2±0.67[x] | 6.2±0.34[a] | 9.1±0.53[c**] |
| Hb[+++] | 12.7±0.23 | 12.7±0.18 | 12.2±0.71 | 12.2±0.33 | 11.2±0.54 | 9.4±0.53[y] | 9.7±0.62 | 10.2±0.66 |

[+] µmole ALA/min/1 erythrocyte, [++] µg ZPP/g Hb, [+++] g/100 ml;
Each figure represents mean ± SE of 6 animals; [x] $p<0.001$, [y] $p<0.05$ versus unexposed control group; [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ versus respective saline group; ** $p<0.01$, * $p<0.05$ versus corresponding normal diet group, as evaluated by the Student's 't' test.

Table 2. Effect of chelators on blood and tissue levels of lead in rats fed normal or vitamin B - complex deficient diet.

| | Unexposed control | Normal diet Pb exposed | | | Unexposed control | Vitamin deficient diet Pb exposed | | |
|---|---|---|---|---|---|---|---|---|
| | | Saline | DMSA | HEDTA | | Saline | DMSA | HEDTA |
| Blood[+] | 4.0±0.29 | 107.2±9.72[x] | 41.7±6.10[a] | 90.3±6.90[c] | 3.6±0.57 | 123.8±20.20[x] | 75.6±8.20[b*] | 97.6±8.10[c] |
| | (6) | (5) | (5) | (5) | (6) | (5) | (5) | (5) |
| Liver[++] | 0.9±0.11 | 9.6±0.80[x] | 2.8±0.30[a] | 4.5±1.40[c] | 1.0±0.11 | 13.3±1.00[x] | 4.6±0.20[a*] | 11.4±0.50[c**] |
| | (6) | (5) | (6) | (6) | (6) | (6) | (5) | (5) |
| Kidney[++] | 2.9±0.36 | 15.4±1.00[x] | 5.6±0.20[a] | 10.1±1.10[c] | 3.1±0.51 | 18.3±1.40[x] | 8.1±0.40[b*] | 10.7±1.10[b] |
| | (6) | (5) | (6) | (5) | (6) | (5) | (5) | (6) |
| Brain[++] | 0.6±0.11 | 2.3±0.20[x] | 2.6±0.30 | 2.9±0.50 | 0.5±0.05 | 3.2±0.30[x] | 3.3±0.60 | 3.8±0.40 |
| | (5) | (5) | (6) | (5) | (5) | (5) | (5) | (6) |

[+] µg/100 ml, [++] µg/g fresh tissue;
Each figure represents mean ± SE of number of animals given in parenthesis; [x] $p<0.001$ versus unexposed control group; [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ versus respective saline group; ** $p<0.01$, * $p<0.05$ versus corresponding normal diet group, as evaluated by the Student's 't' test.

organs and the Pb induced alterations in blood and urinary parameters in rats which were attributed to its constituents interfering with the absorption of Pb in body tissues by forming readily excretable complexes with the circulating Pb (Flora et al. 1984). These observations suggest that the presence of vitamins facilitates the elimination of Pb from the body either by chelating the excess metal or transporting the metal-chelator complex. This may explain the less marked ameliorative effects of DMSA and HEDTA in vitamin deficient animals.

DMSA has been found to be more effective than HEDTA in mobilizing the body Pb and restoring most of the altered urinary and blood biochemical parameters irrespective of the diet fed to the animals. The relative efficacy of the two chelating agents may be related to their nature and chemical structure. Thus, two adjacent, sulfhydryl ( -SH) groups in DMSA apparently provide stronger chelating site for Pb than two amino N in HEDTA (Williams and Halstead 1982). Dithiol chelators containing adjacent -SH groups have been reported to mobilize far more Cd from liver metallothionein and increase Cd excretion than the compounds with non-adjacent -SH groups or a single -SH group (Cherian 1984). Additionally, DMSA like structurally and chemically similar to 2,3 dimercapto-1-propanesulfonic acid (DMPS), may have a better access to intracellular Pb via an anion transport mechanism than HEDTA (Wildenaur et al. 1983). HEDTA may be acting only extracellularly in removing Pb. However, the ineffectiveness of both the chelators in reducing the brain Pb and the alterations in brain biogenic amine levels indicates their inability to cross the blood brain barrier or existing a stronger linkage between Pb and brain bioligands.

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