

EFFECTS OF CHELATION THERAPY ON HEPATIC GLUTATHIONE, LIPID PEROXIDATION AND PHOSPHOLIPID CONTENTS IN LEAD-POISONED RATS

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Abstract : Hepatic lipid peroxidation, glutathione and phospholipid contents of homogenate prepared from the liver of lead-intoxicated male rats treated with 0.3 m mol/kg CaNa_2EDTA and DMSA for 8 weeks, either alone or in combination, were investigated. A significant increase in hepatic malondialdehyde (MDA) and a reduction in glutathione levels was noticed. While a marginal decrease in phosphatidyl choline (PC) level was noticed, no effect on phospholipid contents was seen. Treatment with all the three chelating agents elicited decrease in PC level. DMSA alone was partially effective in restoring lead-induced altered hepatic glutathione and MDA levels. Combined treatment may have an adverse effects on hepatic tissue and does not seem to produce immediate recoveries in the lead-induced hepatic damage.

Key words : lead toxicity chelation treatment hepatic recovery

INTRODUCTION

Conventional therapy for lead poisoning involves administration of calcium disodium ethylenediamine tetra acetic acid (CaNa_2EDTA). This drug has many disadvantages, including high toxicity, redistribution of lead, low therapeutic index, water insolubility and the need for parenteral administration (1, 2). Recently, water soluble meso 2,3-dimercaptosuccinic acid (DMSA) which is an analogue of British Anti Lewisite (BAL) has received considerable attention as suitable replacement for CaNa_2EDTA and has been approved by the US Food and Drug Administration for childhood lead poisoning (3, 4). Flora et al (5) determined the effectiveness of combined treatment with DMSA and CaNa_2EDTA in treating chronic lead poisoning,

which was found to elicit an additive response in depleting lead body burden and restoring lead sensitive biochemical variables. Further, no redistribution of lead to any organ was observed. The present study was planned to find out if the combined treatment produced recoveries in lead induced hepatic damage and if it had any harmful effects on hepatic tissue.

METHODS

Male albino rats (150 ± 10 g) maintained on standard pellet diet (metal contents in ppm dry weight : Cu 10, Mn55, Zn45, Fe70, Co5) and water *ad libitum*. The animals were administered lead as 0.1% lead acetate in drinking water for 8 weeks. Six animals received no treatment and served as control. The lead exposed rats were divided equally into groups of five each and treated for 5 days as follows :

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- Group 1 - Saline
 Group 2 - Ca disodium EDTA, 75 mg/kg, ip once daily
 Group 3 - DMSA, 25 mg/kg, orally, twice daily
 Group 4 - Ca disodium EDTA, as in group 2 + DMSA, as in group 3

The animals were sacrificed by decapitation to collect hepatic tissue.

Standard techniques were employed to determine the level of hepatic glutathione (6), lipid peroxidation (7) and phospholipid contents (8). Lead contents of liver were measured following wet acid digestion with nitric acid (9). Statistical evaluation was done by Student's 't' test.

RESULTS AND DISCUSSION

Table I indicates the effect of Ca disodium EDTA or DMSA either individually or in combination on some hepatic biochemical

variables in lead poisoned rats. Exposure to lead produced marginal depletion in hepatic GSH level and an increase in hepatic lipid peroxidation level. Lead contents also increased significantly on lead exposure. Treatment with CaNa_2EDTA produced a reduction in hepatic GSH level and no effect on lipid peroxidation. On the other hand, treatment with DMSA lead to an increased hepatic glutathione contents, while hepatic MDA level also recovered to the normal level. Both the chelating agents were effective in reducing hepatic lead contents, when given individually. Combined treatment with DMSA and Ca disodium EDTA had an advantage that hepatic MDA level remained unchanged while it produced a significant depletion in hepatic phosphatidylcholine (PC) contents. No effect on sphingomyelin (SPH) or total phospholipid contents were noted. A marginal depletion in hepatic phosphatidyl ethanolamine (PE) contents was noted (Table II).

This study demonstrates lead induced hepatic lipid peroxidation cause a decrease in hepatic GSH concentration which is an important

TABLE I : Effect of chelation treatment on hepatic glutathione, lipid peroxidation and lipid contents in lead poisoned rats.

	Glutathione $\mu\text{g g}^{-1}$	Lipid peroxidation $\text{m mol MDA mg protein}^{-1}$	Lead $\mu\text{g g}^{-1}$
Control animals	6.54 ± 0.21	0.55 ± 0.03	0.61 ± 0.04
Saline	5.35 ± 0.62	0.68 ± 0.03 ^a	6.45 ± 0.04 ^a
CaNa_2EDTA	4.54 ± 0.71 [*]	0.63 ± 0.03	2.23 ± 0.10 [*]
DMSA	6.66 ± 0.25	0.50 ± 0.02 [*]	3.77 ± 0.20 [*]
DMSA + EDTA	5.80 ± 0.16	0.61 ± 0.02	1.77 ± 0.20 [*]

Data of liver lead concentration reproduced from our paper in *Fund Appl Toxicol* with permission from Academic Press. Values are mean ± S.E., n=6.

^a P<0.05 compared to control; ^{*} P<0.05 compared to saline treated (lead exposed).

TABLE II : Effect of chelation on hepatic phospholipids (mg/g) contents in rat.

	PC	PE	SPH	TPL
Control animals	17.82 ± 0.45	8.26 ± 0.17	2.66 ± 0.22	30.87 ± 0.61
Saline	16.45 ± 0.66	7.84 ± 0.54	2.40 ± 0.25	30.47 ± 1.76
CaNa_2EDTA	13.57 ± 0.67 [*]	6.06 ± 1.05	2.55 ± 0.12	30.66 ± 2.06
DMSA	13.53 ± 0.10 [*]	6.56 ± 0.91	3.15 ± 0.09	30.99 ± 0.13
DMSA + EDTA	12.95 ± 1.08 [*]	6.23 ± 0.19 [*]	2.26 ± 0.18	30.94 ± 1.06

Mean ± S.E., n=5.

^{*} P < 0.05 compared to saline (Pb exposed) treated group as evaluated by the Student's 't' test. Abbreviation used : PC-Phosphatidyl choline; PE-Phosphatidyl ethanolamine; SPH-sphingo-myelin; TPL-Total phospholipid.

factor in regulation of lipid peroxidation. Increased GSH concentration induced by DMSA administration protects against lead induced biochemical alterations (5) and lipid peroxidation. Minimal effect on the hepatic total phospholipid contents except for a marked depletion in PC contents could be explained by the fact that during inflammation there could be a change in the phospholipid fraction without any marked change in total phospholipid contents (10). It is also clear that combined treatment does not have any marked effect on lead-induced alterations in hepatic tissue.

The present study thus confirms that

i) combined treatment with a thiol chelator and a soft tissue lead mobilizer like DMSA and a polyaminocarboxylic acid and a hard tissue lead mobilizer like Ca disodium EDTA is more effective than treatment with these chelator individually, however, prolonged treatment with this protocol may lead to certain harmful effects on hepatic organ, ii) A 5 days chelation therapy may not be sufficient in rapid recoveries of lead-induced hepatic biochemical lesions.

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