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Effect of Lead on Lipid Peroxidation in Liver of Rats

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ABSTRACT

The present study was undertaken to understand the biochemical mechanisms of lead toxicity in liver. We observed a significant accumulation of lead in liver following lead treatment, resulting in accentuation of lipid peroxidation. Concomitant to the increase in lipid peroxidation, the activities of antioxidant enzymes, viz., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, were significantly inhibited. A decrease in reduced glutathione with a simultaneous increase in oxidized glutathione was observed following lead exposure, resulting in a reduced GSH/GSSG ratio. These results indicate that lead exerts its toxic effects by enhancing peroxidative damage to the membranes, thus compromising cellular functions.

Index Entries: Lead; liver; lipid peroxidation; antioxidant enzymes; glutathione.

INTRODUCTION

Lead, a toxic heavy metal reputed for its toxic effects on human health, remains a public health hazard. Lead toxicity is reported to be associated with impaired functioning of brain, kidneys, liver, and the hematopoietic system. Dodic et al. *(1)* reported signs of impaired liver function in patients hospitalized for lead poisoning. Lead has been reported to interfere with essential trace element metabolism *(2,3)*, inhibit heme biosynthesis *(4)*, and alter Ca^{2+} homeostasis *(5)*. Recently, the primary effects of lead were thought to be mediated by damage to cell

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membranes. A pathological free radical mechanism that leads to lipid peroxidation and degradation of phospholipids with loss of membrane integrity is currently considered an important factor in the development of organ damage *(6)*. Quinlan et al. *(7)* demonstrated enhanced iron dependent lipid peroxidation in liposomes, erythrocytes, and rat liver microsomes by lead in vitro. Furthermore, Cook et al. *(8)* observed alterations in membrane phospholipid composition and microviscosity in erythrocytes of lead-exposed workers. Therefore, the effect of lead on lipid peroxidation was investigated in the present study in order to determine whether peroxidative damage is the initial event responsible for damage to hepatocytes.

MATERIALS AND METHODS

Animals and treatment

Male albino Wistar rats, weighing between 100 and 110 g, were used throughout the experiments. The animals were housed in polypropylene cages under hygienic conditions and were fed rat pellet diet (Hindustan Lever Ltd., India) and water ad libitum. The animals were divided into following two groups:

Control Group

This group consisted of six rats that were given normal saline intragastrically for the duration of the treatment.

Lead-Treated Group

This group consisted of eight rats that were given lead as lead acetate at a dose of 50 mg/kg body wt, intragastrically, for a period of 8 wk.

Dissection and Homogenization

After a period of 8 wk, animals were fasted overnight and killed by decapitation, the livers removed, and rinsed in ice-cold isotonic saline. A 10% (w/v) tissue homogenate was prepared in 0.1*M* Tris-HCl, pH 7.4. The postnuclear fraction of catalase assay was obtained by centrifugation of the homogenate at 1000*g* for 20 min at 4°C and for other enzyme assays centrifuged at 12,000*g* for 60 min at 4°C.

Lipid Peroxidation

The quantitative measurement of lipid peroxidation was performed according to the method of Wills et al. *(9)*. The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as nmol MDA/mg protein using the molar extinction coefficient of MDA–thiobarbituric chromophore ($1.56 \times 10^5/M/cm$).

Enzyme Assays

Superoxide dismutase was assayed according to the method of Martin et al. *(10)*; the autoxidation of hematoxylin to hematin was inhibited by the addition of the enzyme. The activity of the enzyme was expressed as U/mg protein, where 1 U of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%. Catalase activity was assayed according to the method of Luck *(11)*, wherein the breakdown of H_2O_2 is measured at 240 nm. The results were expressed as nmol H_2O_2 decomposed/min/mg protein.

Glutathione peroxidase was assayed by the method of Lawrence and Burk *(12)*, using H_2O_2 as the substrate. The enzyme activity was expressed in terms of nmol NADPH oxidized/min/mg protein.

Glutathione reductase activity was measured by the method of Horn *(13)*; the reduction of oxidized glutathione to reduced glutathione was measured at 340 nm. The results were expressed as nmol NADPH oxidized/min/mg protein.

Other Parameters

Glutathione

Reduced glutathione was estimated by the method of Ellman *(14)* and oxidized glutathione by the method of Srivastava and Beutler *(15)*. Total sulfhydryl groups were estimated according to the method of Sedlak and Lindsay *(16)* using Ellman's reagent.

Metal Analysis

Tissues were analyzed for lead content by the wet acid digestion method of Evenson and Anderson *(17)* and analyzed with a Direct Current Plasma Emission Spectrophotometer (Beckman, Fullerton, CA). The results were expressed as μg lead/g tissue.

Zinc protoporphyrin was measured in heparinized rat blood on hematofluorimeter Model 206 (AVIV). The results were expressed as μmol zinc protoporphyrin/mol heme.

Proteins were estimated in the samples by the method of Lowry et al. *(18)*. Statistical analysis was employed. Values in the tables are mean ± SD. Comparisons between the means of lead treated and control rats were made using the student's *t*-test. Values with $p < 0.05$ were considered significant.

RESULTS

Lead exposure (50 mg/kg body wt), intragastrically, for a period of 8 wk resulted in a significant accumulation of lead and zinc protoporphyrin, a peripheral marker of lead toxicity in the blood of lead-treated rats (Fig. 1). The data in Table 1 indicate that lead accumulated signifi-

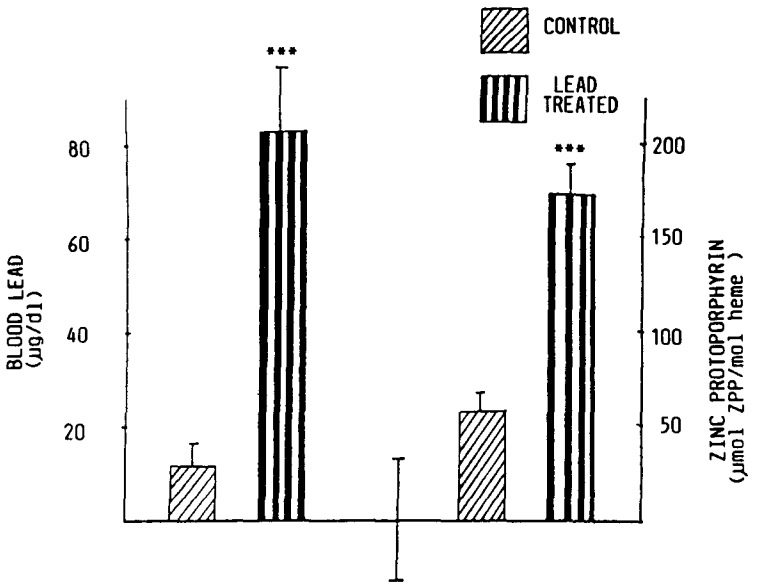

Fig. 1. Zinc protoporphyrin and blood lead levels in blood of control and lead-treated animals. Values are mean ± SD of six to eight animals/group. *p, Significantly different from control group, ***$p < 0.001$.

Table 1
Effect of Lead Exposure on Lead Accumulation and Lipid Peroxidation in Liver of Rats[a]

	Control	Lead-Treated
Lead, µg/g tissue	0.14 ± 0.01	18.43 ± 2.11[b]
Lipid peroxidation, nmol MDA/mg protein	1.24 ± 0.05	1.92 ± 0.13[b]

[a]Animals were exposed to lead at a dose of 50 mg/kg body wt for a period of 8 wk, intragastrically. Results are mean ± SD of six to eight animals/group.
[b]$p < 0.001$, significantly different from control group.

cantly in livers of lead-treated rats during the course of treatment, with a concomitant increase in lipid peroxidation.

The increase in lipid peroxidation was accompanied by a marked decrease in the activities of enzymes, e.g., superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase (Table 2). Further, the glutathione status of rats following lead administration was also affected, resulting in a reduction in the levels of reduced glutathione and a concomitant increase in the levels of oxidized glutathione (Table 3). However, the levels in the total hepatic sulfhydryl groups were increased significantly following lead exposure.

Table 2
Effect of Lead Exposure on Antioxidant Enzymes in Liver of Rats[a]

	Control	Lead-Treated
Superoxide dismutase, U/mg protein	12.01 ± 0.18	6.42 ± 0.21[b]
Catalase, μmol H_2O_2 decomposed/min/mg protein	177.86 ± 12.72	135.46 ± 17.29[b]
Glutathione peroxidase, nmol NADPH oxidized/min/mg protein	310.96 ± 9.99	246.03 ± 16.85[b]
Glutathione reductase, nmol NADPH oxidized/min/mg protein	115.41 ± 9.15	78.97 ± 9.08[b]

[a]Animals were exposed to lead at a dose of 50 mg/kg body wt for a period of 8 wk, intragastrically. Results are mean ± SD of six to eight animals/group.
[b]$p < 0.001$, significantly different from control group.

Table 3
Effect of Lead Exposure on Glutathione Status in Liver of Rats[a]

	Control	Lead-Treated
GSH, μmol/mg protein	47.18 ± 0.90	41.12 ± 1.37[b]
GSSG, μmol/mg protein	1.08 ± 0.11	3.47 ± 0.82[b]
Total sulfhydryl groups, μmol/mg protein	112.03 ± 3.39	195.29 ± 11.61[b]

[a]Animals were exposed to lead at a dose of 50 mg/kg body wt for a period of 8 wk, intragastrically. Results are mean ± SD of six to eight animals/group.
[b]$p < 0.001$, significantly different from control group.

DISCUSSION

Our results demonstrate that lead exposure results in a significant increase in lipid peroxidation, a process of oxidative deterioration of membrane polyunsaturated fatty acids with a loss of membrane phospholipids, and, finally, of membrane integrity. The results obtained are in agreement with those of Quinlan et al. *(7)*, who reported enhanced iron-dependent lipid peroxidation in liposomes, erythrocytes, and liver microsomes by in vitro lead. However, peroxidative damage by lead is not the only mechanism attributed to lead toxicity. Reports are available indicating the interference of lead with essential trace element metabolism *(2,3)*, heme biosynthesis *(4)*, and Ca^{2+} homeostasis *(5)*.

The ability of lead to enhance lipid peroxidation may be attributed to the indirect effect of lead on free radical scavenging enzymes and not to a direct effect of lead on lipid peroxidation, as lead does not partici-

pate in the oxidation reduction cycle. Recently, Oteiza and Bechara *(19)* demonstrated that lead-enhanced lipid peroxidation might result from enhanced levels of 5-amino levulinic acid, which accumulates after lead exposure.

The increase in lipid peroxidation was accompanied by a simultaneous decline in the activities of antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and catalase. The decline in activity of the various antioxidant enzymes may result from the interaction of lead with sulfhydryl groups or the interaction with essential metal cofactor in these enzymes and molecules. Mylorie et al. *(20)* observed a decline in the activity of superoxide dismutase and suggested that the decline may result from lead-induced copper deficiency. Schrauzer et al. *(21)* demonstrated antagonistic effects between lead and selenium, leading to reduced selenium uptake that may affect the glutathione–peroxidase activity, which requires selenium as a cofactor. The reduction in the catalase activity may result from the inhibition of heme biosynthesis by lead *(22)*, leading to reduction in catalase activity.

Glutathione, an important antioxidant, which protects the membrane from oxidative insult, is thus considered a critical determinant for the threshold of tissue injury caused by environmental chemicals *(23)*. The levels of reduced glutathione decreased significantly following lead exposure, with a significant accumulation of oxidized glutathione, resulting in reduced GSH/GSSG ratio. The alteration in glutathione status might either result from reduced glutathione reductase activity or utilization of glutathione in the detoxification of lead. The altered glutathione status may ultimately affect the thiol: disulfide ratio of cellular proteins and ultimately affect their functioning.

Interestingly, we observed a marked increase in total sulfhydryl groups in liver. This increase may be attributed to a possible induction of metallothionein by lead. However, the role of metallothionein in the detoxification of lead ions has not been reported.

The results reported here indicate that the lead-exposed population show enhanced peroxidation of membrane lipids, which may have dramatic consequences on cell membrane, ultimately affecting membrane proteins, enzymes, receptors, and ion channels.

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