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PREVENTIVE ACTION OF ZINC AGAINST LEAD TOXICITY

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Abstract: The study was aimed to assess the protective efficacy of zinc against hemo and hematotoxicity induced by lead. Two groups of 8 rats each, were administered lead acetate 20 mg/kg bw (ip) for 3 days. One group in addition was injected 5 mg/kg bw (ip) zinc acetate for next three days. A third group of 8 rats was given three injections of normal saline and served as control. All the animals were sacrificed on eighth day and assessed for hematological changes, heme synthesizing pathway enzymes, hepatic drug metabolizing status and sulfhydryl levels in blood and liver. Lead administration resulted in decreased hemoglobin, increased reticulocytosis, depression of delta aminolevulinic acid dehydratase (ALAD) and uroporphyrinogen I synthetase (UPS) activity in blood and liver. *In vitro* metabolism of drugs aminopyrine, aniline and p-nitroanisole by liver homogenate and *in vivo* metabolism of pentabarbitone was also reduced in lead exposed rats. Zinc treated rats showed improved hematological profile and activated ALAD and UPS activity, recovery of N-demethylation of aminopyrine and O-demethylation of p-nitroanisole and partial restoration of free thiol levels in blood and liver thereby indicating that zinc could confer protection against lead toxicity.

Key words: lead zinc hemotoxicity drug metabolism

INTRODUCTION

Chelation therapy is often used for heavy metal poisoning but it is inherent with serious side effects. Protection by dietary means is a comparatively recent approach. The rationale behind it is that the toxic chemicals can interact with other dietary constituents or induce nutritional deficiencies due to their greater utilization.

One of the most studied and the best documented effects of lead toxicity is inhibition of heme synthesis and most of the studies on dietary components with the protective/ prophylactic efficacy against lead toxicity have centered around reversal and reactivation of the enzymes involved in heme metabolism (1-3). But inhibition of heme synthesis may limit the supply of heme for its incorporation into hemoproteins other than hemoglobin, such as myoglobin, catalase, mitochondrial and microsomal cytochromes and hence heme related functions like mitochondrial respiration and microsomal drug metabolising activity may also get affected by lead toxicity. Lead induced inhibition of drug metabolising enzymes accompanied by dimunition of cytochrome P450 and b5 in several studies provide sufficient evidence for this (4-7). Based on prolongation of hexabarbital sleeping time and zoxazolamine paralysis time in rats, slowed elimination of these drugs from the body has been suggested in lead exposed rats (8). On the other hand, zinc is known to have a direct inducing action on heme syntheisis (9) and subtoxic doses of zinc in drinking water have been reported to shorten hexabarbital sleeping time and increase ethylmorphine N-demothylase activity and cytochrome P450 (10). Further, it has been documented that several of the enzymes of heme biosynthetic pathway get depressed

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during zinc deficiency (11). Further, plasma zinc levels were found to be about 20% lower among childern who had substantially elevated lead levels (12).

Keeping in view the above findings, the present study on influence of zinc on lead induced alterations in heme synthesizing enzymes and its implication on hepatic drug metabolizing enzymes, could gainfully explain therapeutic efficacy of zinc in treatment of lead toxicity.

METHODS

Male albino rats, weight of 240 to 260 g were used in this study. The animals were housed in polypropylene cages with rice husk as the grounding matrix. Free access to food and water was allowed. The food was pelleted diet supplied by Lipton (India) Ltd.

A group of 16 rats was treated with lead acetate 20 mg/kg bw (ip) once daily for three consecutive days and the other 8 with injectable sterile normal saline. Lead administered rats were divided in two groups of 8 each. One group was injected zinc acetate, 5 mg/kg bw (ip) once daily for next 3 days and the other left undisturbed. The animals were fasted overnight and on the seventh day mildly anesthetized with anesthetic ether. Body cavity was opened and blood was collected from the heart. The livers were removed quickly, minced and homogenized in five volumes of 0.1 M phosphate-buffer, pH 7.4 containing 1.15% KCl in a Potter-Elvehjem homogeniser. Homogenates were centrifuged at 9000 x g in a refrigerated centrifuge at 4°C for 10 min. Enzyme assays were performed in the supernatant fraction obtained as above within 6 hrs after the animals were sacrificed. δ-Aminolevulinic acid dehydratase (EC 4.2.1.24) activity was measured in whole blood and liver preparations by the spectrophotometeric method of Burch and Siegel (13). Uroporphyrinogen I synthetase (EC 4.1.1.37) was assayed by modified method of Levin and Coleman (14) as described by Woods (15). Aniline hydroxylase activity was determined by a method described by Imai et al, (16). The O-demethylation of p-nitroanisole was measured using the procedure developed by Neal and Dubois (17) and N-demethylation of aminopyrine by Cochine and Axelrod (18). Cytochrome P450 and b5 were determined in $CaCl_2$ sedimented microsomes suspended in phosphate buffer 0.1M pH 7.4 by the method of Omura and Sato (19). Protein was assayed by the method of Lowry et al, (20) and total, free and bound thiol content in blood and liver by Sedlak and Lindsay procedure (21) Data was analysed by Student's 't' test.

RESULTS

It is evident from Table I that lead exposure caused a slight decrease in body weight and increase in liver weight, thereby resulting into a significant increase in relative liver weight. Liver protein, microsomal protein and liver DNA were significantly lower than control rats ($P<0.05$, $P<0.01$ and $P<0.001$ respectively). In contrast zinc treated rats did not show any appreciable change in body weight, liver weight and DNA content. As compared to lead exposed rats protein concentration in liver was slightly higher in zinc treated rats but as compared to control rats it was decreased. Hematological indices in lead exposed rats indicated a marked degree of reticulocytosis (significance $P<0.01$) and significantly reduced levels of hemoglobin ($P<0.01$) while zinc treated rats showed somewhat improved but nonetheless similar hematological profile.

Effect of zinc on blood ALAD, UPS and microsomal enzyme activity in lead poisoned rats is shown in Table II. ALAD and UPS activities were significantly lower in blood as well as liver of lead administered rats as compared to that of control animals ($P<0.001$). Treatment with zinc improved ALAD and UPS activities to a significant extent as compared to lead exposed animals as is evident by 127.9% higher ALAD activity in blood ($P<0.001$), 107.5% higher activity in liver ($P<0.01$) and 280.3% ($P<0.01$) and 150.2% ($P<0.01$) increased UPS activity in blood and liver respectively as

TABLE I : Body weight, liver weight, liver protein, liver DNA and hematological indices in rats poisoned with lead and treated with zinc.

Parameter	*Control*	*Lead*	*Lead + Zinc*
Body Weight (g)	247.20 ± 7.30	231.70 ± 6.20	240.00 ± 10.40
Liver Weight (g)	7.39 ± 0.38	7.88 ± 0.33	7.39 ± 0.41
Relative liver Weight (g/100 g)	2.98 ± 0.10	3.39 ± 0.09**	3.05 ± 0.10
Liver Protein (mg/g wet wt.)	193.78 ± 7.79	169.96 ± 5.85*	175.89 ± 5.11
Microsomal Protein (mg/g wet wt.)	17.94 ± 1.94	12.60 ± 1.14***	—
Liver DNA (mg/g wet wt)	9.56 ± 0.28	8.22 ± 0.25**	9.17 ± 0.17
Hemoglobin (g/dl)	16.61 ± 0.65	13.38 ± 0.40***	14.21 ± 0.44***
Hematocrit (%)	48.59 ± 3.26	51.82 ± 2.17	51.22 ± 2.30
MCHC	35.96 ± 3.04	33.06 ± 2.47	34.20 ± 2.58
Reticulocytes (%)	1.283 ± 0.197	3.157 ± 0.308***	2.115 ± 0.039***

Values are mean ± SEM of 8 animals.
Astrisks indicate significance level ***P<0.001; **P<0.01 and *P<0.05

TABLE II : Effect of 5 mg/kg/bw zinc actate on heme synthesis and drug metabolism in rats poisoned with 20 mg/kg bw lead acetate.

Enzyme Source	*Control*	*Lead*	*Lead + Zinc*
	δ-Amino Levulinic Acid Dehydratase (nmoles PBG formed/60 min/ml blood or/mg of liver protein)		
Blood	168.31 ± 22.16	69.14 ± 8.30***	157.55 ± 11.02
Liver	13.30 ± 0.72	5.48 ± 0.56***	11.37 ± 1.57
	Uroporphyrinogen I Synthetase (nmoles PBG utilized/60 min/100 ml of blood or/mg of liver protein)		
Blood	31.14 ± 3.72	15.88 ± 2.15***	60.38 ± 11.71*
Liver	10.37 ± 0.72	3.01 ± 0.45***	7.53 ± 1.17**
	Aniline Hydroxylase (nmoles of p-aminophenol formed/min/mg of protein)		
Liver	84.74 ± 11.51	69.61 ± 5.91	55.41 ± 5.54*
	Aminopyrine N-demethylase (nmoles of HCHO formed/min/mg of protein)		
Liver	267.28 ± 58.30	198.85 ± 33.80	217.98 ± 24.57
	p-Nitroanisole O-demethylase (nmoles of p-nitrophenol formed/min/mg of protein)		
Liver	25.78 ± 3.39	17.22 ± 3.93	40.16 ± 5.72*
	Cytochrome P450 (nmoles/mg of protein)		
Liver	5.05 ± 0.50	3.59 ± 0.38*	4.45 ± 0.39
	Cytochrome b5 (nmoles/mg of protein)		
Liver	5.50 ± 0.35	5.17 ± 0.33	5.35 ± 0.40

Values are mean ± SEM of 6-8 animals.
Astrisks indicate significance level ***P<0.001; **P<0.01 and *P<0.05

compared to lead treated group. Activities of drug metabolising enzymes, aniline hydroxylase, aminopyrine N-demethylase and p-nitroanisole O-demethylase were also reduced significanlty in lead treated rats (P<0.05). Zinc could restore, though partially aminopyrine N-demethylation and p-nitroanisole O-demethylation. Aniline hydroxylation, on the contrary, was further depressed be zinc treatment. Microsomal cytochrome P450. levels in lead exposed animals were significanlty lower (P<0.05) but in zinc treated rats decrease was of lesser magnitude.

Sleeping time after a dose of pentabarbitone was significanlty prolonged in lead exposed rats (P<0.02) and loss of rightening reflex much quicker whereas in zinc exposed rats, loss of rightening reflex was slower and sleeping latency lower (Table III).

TABLE III : Sleeping time in rats poisoned with lead and treated with zinc.

Time in min	*Control*	*Lead*	*Lead + Zinc*
Loss of rightening	2.43 ± 0.13	2.17 ± 0.11	3.00 ± 0.22*
Gain of rightening	115.57 ± 22.73	199.53 ± 18.61**	161.63 ± 31.05
Slepping	113.13 ± 22.80	197.38 ± 18.55**	158.63 ± 31.05

Values are mean ± SEM of 6 animals.
Astrisks indicate significance level **P<0.02; *P<0.05

TABLE IV : Effect of zinc on total and non-protein sulfhydryl concentration in blood and liver of rats poisoned with lead.

Source	*Control*	*Lead*	*Lead+Zinc*
Blood			
Total SH	285.54 ± 5.47	133.79 ± 3.14***	179.96 ± 9.79***
NPSH group	8.06 ± 0.11	2.32 ± 0.15***	7.68 ± 0.18
Liver			
Total SH	1032.70 ± 65.38	855.26 ± 22.35*	795.97 ± 21.82*
NPSH group	830.40 ± 13.38	759.20 ± 27.73**	

The concentration of sulfhydryl groups is expressed in nmoles/dl for blood and in umoles/g of tissue for liver.
Values are mean±SEM of 6-8 animals.
Astrisks indicate significance level ***P<0.001; **P<0.01; and *P<0.05

Lead administration caused an acute deficiency of total and free thiol status in blood as well as liver (Table IV). Zinc treatement could restore to some extent circulating levels of total and free sulfhydryl groups but at the cost of liver thiols which were further reduced by 23% and 21% on treatment with zinc.

DISSCUSSION

Liver enlargement (as indicated by significantly elevated liver : body weight index) accompanied by reduction in macromolecular content indicated necrotic alterations due to lead toxicity. Reduction in hemoglobin could be due to increased destruction of red blood cells hence stimulating erythropoesis as indicated by increased reticulocytosis. Supplementation with zinc reduced these toxic symptoms to some extent.

In conformation with the results of earlier studies (5, 22, 23), the present investigation too demonstrated that lead inhibited heme synthesizing enzymes ALAD and UPS. The reduced availability of heme resulting thereby may interfere in its incorporation in various heme compounds, hemoglobin and cytochrome P450 which in turn could be the cause of anemia and reduced drug metabolizing capacity of liver as reflected by impaired metabolism of aminopyrine, aniline, p-nitroanisole and pentabarbitone (longer sleeping times). In fact, both cytochrome P450 and b5 levels were reduced in lead exposed rats in this study and hemoglobin level too was less. Treatment with zinc to lead exposed rats not only restored ALAD and UPS in erytheroid and hepatic cells and mean cell hemoglobin concentration in red blood cells but also prevented inhibition of O-demethylation of p-nitroanisole and N-demethylation of aminopyrine thus giving ample reason to speculate that lead induced zinc deficiency could be the cause of inhibition of heme synthesis which in turn impaired drug metabolism and supplementation by zinc automatically restored both. Lead has been reported to reduce tissue zinc levels (24) and supplementation with zinc, reduces absorption, retention and toxic action of lead (1).

Mechanism of reactivation of δ-ALAD by zinc is now well established. Its 28 sulfhydryl groups make it readily susceptible to mercaptide formation by many heavy metals including lead and its allosteric nature renders it to conformational deactivation by lead (25, 26). Raising the concenteration of zinc by exogenous supplementation is probably able to protect essential sulfhydryl groups of the enzyme by conformational change or prevent and even replace lead from binding to the active sites of ALAD and is able to reactivate it and thus restoring supply of heme for various requirements including drug metabolism. However, further inhibition of aniline hydroxylase by zinc treatment could not be explained by this reasoning. There could be some other explanation such as differential substrate affinity of these mono-oxygenases in the presence of two types of metal ions or differential requirement of individual mono-oxygenases for various factors such as vitamin C or free thiols. Affinity of lead for sulfhydryl groups is well known and it has been suggested that apart from enzymes of heme synthetic pathway, lead might inhibit flavin containing mono-oxygenase, NADPH cytochrome C reductase and cytochrome P450 reductase by blocking the thiol groups (26). Indeed, in this study zinc supplementation to lead administered rats could restore free sulfhydryl status in liver, though partially, whereas it failed to confer any beneficial effect on total sulfhydryl levels and protein bound sulfhydryl levels. Induction of metallothioneins by zinc could be another mechanism of protection against lead toxicity.

Based on the present results, therapeutic importance of zinc treatment against lead toxicity could be envisaged but with a word of caution since at higher dosages zinc can even exacerbate the toxic effects of lead. Further experimentation is required to arrive at an optimal dose effect relationship to derive maximal benefit out of this antagonistic metal-metal interaction.

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