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Immunomodulation following zinc supplementation during chelation of lead in male rats

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Influence of zinc supplementation (30 and 45 mg kg^{-1}, orally once for 5 days) during chelation of lead (0.3 mmol kg^{-1}, chelating agent, i.p., once for 5 days) on some selected variables of the immune system was investigated in male rats. Treatment with $CaNa_2EDTA$ either alone or in combination with zinc (30 mg kg^{-1}) produced a significant recovery in lead induced alteration in primary antibody forming cells to T-dependent antigen and the delayed-type hypersensitivity response to bovine albumin. However, biologically significant recovery was observed only with zinc at a dose of 45 mg kg^{-1}. It is assumed that zinc depletion during lead exposure and chelation treatment lead to harmful effects on cellular proliferation by inhibiting DNA synthesis and various enzymes during mitosis. The zinc supplementation fulfills this requirement during proliferation and clonal expansion of immunocompetent cells augmenting the immune system.

Keywords: chelation, immune system, lead exposure, rat, zinc depletion

Introduction

Lead is a known environmental contaminant and its immunotoxic effects have been well demonstrated (Faith *et al*. 1979, Blakley & Archer 1981, Luster & Blank 1987). Extensive use of EDTA or $CaNa_2EDTA$ for the management of lead poisoning may lead to disturbances in the immune system due to excessive loss of endogenous zinc (Cantilena & Klaasen 1982). The depletion of zinc is known to induce biochemical, morphological and immunological alterations (Flora & Kumar 1991). Safe and efficient therapy for the treatment of lead poisoning using $CaNa_2EDTA$ and concomitant zinc supplementation have been reported (Thomas & Chisolm 1985, Flora & Tandon 1990). Although, Zn–$CaNa_2EDTA$ therapy was successful in reversing various biochemical effects and lead uptake, whether it also augments immune system recovery needs to be evaluated. The present study was therefore planned to delineate the dose-dependent effects of zinc supplementation on immune system recovery during chelation therapy of lead poisoning.

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Materials and methods

Animals

Wistar male rats weighing 120 ± 10 g were employed in the study. Animals were maintained on sterile dust free rice husk, and given food and water *ad libitum*.

Treatment

The animals were divided into five groups consisting of 10 rats in each. The first group of animals (normal control) received sodium acetate (6.3 mmol kg^{-1}). The remaining animals received lead as lead acetate (6.3 mmol kg^{-1}) in drinking water for 8 weeks. Later, lead exposed animals were treated either with normal saline (group II), $CaNa_2EDTA$ (0.3 mmol kg^{-1}; Group III), $CaNa_2EDTA$ and zinc (30 mg kg^{-1}; Group IV) or $CaNa_2EDTA$ and zinc (45 mg kg^{-1}; group V).

Zinc was given as zinc sulfate orally and EDTA was injected i.p. Animals were treated for seven consecutive days and subjected to immunological studies on day 8 of the treatment. Relative spleen and thymus weight, antibody forming cell (AFC) response to sheep red blood cells

(SRBC) and the delayed-type hypersensitivity (DTH) response to bovine albumin (BSA) were determined.

AFC to SRBC

Animals were immunized with 5×10^8 SRBC (i.p.) on day 3 of the chelation therapy and five animals from each group were sacrificed 24 h post the last day of the therapy. Spleens were separated, single cell suspensions were prepared and later splenocytes were employed for AFC determinations as described by Cunningham (1965).

DTH response to BSA

Animals were sensitized s.c. with 100 μg BSA in complete Freund's adjuvant (CFA) on day 8 post-treatment at the base of the tail and later challenged with 75 μg of heat aggregated BSA on day 7 post-sensitization. Animals were challenged intra-dermally with BSA in the left footpad and the right footpad received saline. Footpad swelling was measured in both the hind legs 24 h post-challenge using calipers (Mitutoyo, Japan). The DTH was determined by subtracting the footpad swelling of the right footpad from the values of the left and expressed as mean millimeter footpad swelling ± SEM (Exon *et al.* 1989).

Statistical analysis

All results were expressed as mean ± SE and the statistical significance was determined using Student's *t*-test.

Results and discussion

Lead exposure produced significant immunosuppression in rats as evident from the changes in relative organ (spleen and thymus) weight, DTH response to BSA and AFC response to SRBC which were found to be significantly suppressed as compared with controls (Tables 1 and 2).

The treatments with $CaNa_2EDTA$ or Zn–$CaNa_2EDTA$ supplementation were evaluated in terms of immune system recovery. The zinc supplementation (30 and 45 mg kg^{-1}) produced a more significant increase in the recovery to both AFC and DTH response than in the saline treated animals. Treatment with $CaNa_2EDTA$ alone induced only marginal recovery as compared with therapy with zinc and $CaNa_2EDTA$. The maximum recovery in AFC (93.5%) and DTH (86.25%) was observed with zinc at 45 mg kg^{-1} given along with $CaNa_2EDTA$ therapy.

Relative spleen and thymus weight remained insignificant to both $CaNa_2EDTA$ or with concomitant zinc supplementation. As indicated in Table 1, $CaNa_2EDTA$ alone produced only a marginal recovery in relative spleen (14%) and thymus (13%) weights. This recovery is found to be statistically significant; however, zinc supplementation indicated

Table 2. Influence of zinc supplementation during chelation of lead by $CaNa_2EDTA$ on AFC and the DTH response in rats

Treatment	AFC per 10^6 splenocytes	DTH (mean mm foot pad swelling)
Normal animals	936 ± 35.9	4.0 ± 0.17
Lead exposed (control)	644 ± 28.5[a]	1.41 ± 0.05[a]
$CaNa_2EDTA$	740 ± 19.5[b]	2.55 ± 0.10[b]
Zn (30 mg kg^{-1}) + $CaNa_2EDTA$	798 ± 13.4[b]	3.04 ± 0.13[b]
Zn (45 mg kg^{-1}) + $CaNa_2EDTA$	876 ± 23.5	3.45 ± 0.18

Values are mean ± SE, $N = 5$.
[a] $P < 0.001$, [b] $P < 0.01$ compared with normal animals as evaluated by Student's *t*-test.

Table 1. Influence of zinc supplementation during chelation of lead with $CaNa_2EDTA$ on relative organ (spleen and thymus) weight of rat

Treatment	Body weight (g)	Relative organ weight[a]	
		spleen	thymus
Normal animals	213.6 ± 9.99	0.461 ± 0.012	0.171 ± 0.005
Lead exposed (control)	200.4 ± 7.81	0.306 ± 0.023[b]	0.085 ± 0.007[b]
$CaNa_2EDTA$	220.4 ± 9.30	0.395 ± 0.023[c]	0.149 ± 0.028[d]
Zn (30 mg kg^{-1}) + $CaNa_2EDTA$	226.2 ± 9.50	0.370 ± 0.020[c]	0.149 ± 0.005[d]
Zn (45 mg kg^{-1}) + $CaNa_2EDTA$	220.6 ± 6.50	0.336 ± 0.013[b]	0.146 ± 0.007

Values are mean ± SE; $N = 5$.
[a] Relative organ weight = (organ weight/body weight) × 100.
[b] $P < 0.001$, [c] $P < 0.01$, [d] $P < 0.05$ compared with normal animals as evaluated by Student's *t*-test.

even less and statistically insignificant recovery in these variables (Table 1).

Treatment of lead poisoning with $CaNa_2EDTA$ has been shown to induce biochemical (Flora & Kumar 1993) and morphological alterations (Planas-Bohne & Lohbrier 1976, Rosenblatt and Aronson 1978). These toxic effects are attributed to depletion of endogenous trace metals, particularly zinc (Thomas & Chisolm 1986, Brownie 1986). This investigation indicated a dose-dependent effect of zinc supplementation on immune system recovery during chelation of lead. The zinc supplementation at 30 and 45 $mg\,kg^{-1}$ indicated beneficial effects on recovery of functional parameters like AFC and the DTH response. However, relative spleen and thymus weight recovery was found to be biologically insignificant. In a recent review, these apical parameters were shown to be less sensitive, but we deduced in this study that the brief period of 7 days for organ weight recovery may not be sufficient. It is assumed that recovery in these parameters may take more time after the termination of therapy.

Treatment with $CaNa_2EDTA$ produced significant recovery in AFC response to T-dependent antigen SRBC and DTH response to BSA, but it was only marginal. Oral zinc supplementation during chelation ($CaNa_2EDTA$) augmented recovery in both AFC and the DTH response. Zinc supplementation at 30 $mg\,kg^{-1}$ could give only 54% recovery in AFC response, whereas the DTH response recovery was only marginal. However, 45 $mg\,kg^{-1}$ zinc supplementation induced maximum recovery in both AFC and the DTH response in lead intoxicated rats.

Since EDTA is a non-specific metal chelating agent, it leads to depletion of many important trace elements including endogenous zinc (Cantilena & Klaassen 1982, Victery *et al*. 1986). Zinc is reported to be an important component for DNA synthesis, metalloenzymes, DNA polymerase, RNA polymerase and thymidine kinase (Brownie *et al*. 1986).

In the present study zinc supplementation during chelation with $CaNa_2EDTA$ indicated beneficial effects on immune system recovery. It is considered that zinc depletion due to $CaNa_2EDTA$ chelation therapy leads to inhibitory effects on immunocompetant cells undergoing activation, differentiation and clonal expansion into activated T or antibody secreting B cells. The concomitant zinc supplementation restores these biological processess and augments the recovery of the immune system. Furthermore, our study indicates that a higher dose (45 $mg\,kg^{-1}$) conferred more beneficial effects. Flora *et al*. (1991) reported decreased absorption of lead in the presence of zinc alone or with methionine. The beneficial effects of zinc as a dietary supplement have been found to be effective in reducing lead induced toxicity (Papaioannou *et al*. 1978, Flora *et al*. 1991).

In summary, our results suggest that apart from the general toxicity induced by lead, the immunotoxic effects can also be prevented using EDTA therapy. However, effects induced by depletion of zinc on the immune system could be reversed by zinc supplementation during chelation therapy of lead.

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