Effect of antioxidant ascorbic acid, l-methionine or α-tocopherol alone or along with chelator on cardiac tissue of lead-treated rats

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ABSTRACT

An experiment was conducted using 42 IVRI 2CQ rats to evaluate the effects of three antioxidants, ascorbic acid, l-methionine or α-tocopherol alone, or chelator CaNa₂EDTA alone or along with antioxidant α-tocopherol, on lead accumulation, status of lipid peroxidation, and of copper and zinc concentration in cardiac tissue of lead-treated rats. Lead was given intraperitoneally as 1% lead acetate solution at the dose rate of 1mg of Pb²⁺/kg body mass for a period of 30 days. The lead was then withdrawn and the lead-exposed rats (n=36) were randomly divided into six groups, six lead-treated rats in each group. A further six rats were given no treatment, including lead exposure, to serve as negative controls. The rats were sacrificed under light anaesthesia one day after one week of treatment with antioxidant ascorbic acid, l-methionine or α-tocopherol or with chelator CaNa₂EDTA alone or along with antioxidant α-tocopherol. Blood samples were collected and heart was quickly excised. Mean lead concentration in cardiac tissue was significantly higher in the lead-treated group, even after its withdrawal for a period of seven days (5.02 ± 1.06 vs. 0.40 ± 0.09 µg/gm). The treatment with chelator plus antioxidant α-tocopherol lowered the cardiac lead burden but the level remained significantly higher than that of the negative control. There was a non-significant increase in lipid peroxide levels in the cardiac tissue of lead-exposed untreated rats and either of the antioxidants lowered the lipid peroxide level, but the differences between the different treatment groups remained statistically comparable at P>0.05. The mean concentration of copper and zinc in cardiac tissue remained statistically comparable among the different treatment groups.

Key words: lead, antioxidant, chelator, rat, heart

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**Introduction**

Lead is a common industrial and environmental pollutant. Prolonged exposure at a sub-lethal dose to this toxicant is considered to be a risk factor for cardiovascular diseases. It leads to elevated arterial pressure, alteration in the cardiac conduction system and degenerative changes of cardiac musculature, swelling of the Purkinje fibers and decreased contractility (ASHOKAN, 1974; DEY et al., 1993; TSAO et al., 2000). Recent studies (BENER et al., 2001; VAZIRI et al., 2001) revealed that lead-associated hypertension was due in part to increased generation of reactive oxygen species (ROS), particularly increased hydroxyl radical production, suggesting that lead-induced cardiac damage may occur as a consequence of the propensity of lead to disturb the delicate pro-oxidant and antioxidant balance in the cardiac tissue.

Lead accumulates in almost every tissue, including heart, following experimental feeding (SINGH et al., 1976; FICK et al., 1976). Conventional therapeutic management includes chelation of lead from accumulated tissue. However, recent implication of oxidative stress contributing to lead-associated tissue injury suggests incorporation of antioxidants for better therapeutic management (HALLIWELL, 1994; PATRA et al., 2001). Use of antioxidants in vitro proved beneficial in protecting cells from oxidative damage (ERCAL et al., 1996). The present study evaluates the lead burden and status of lipid peroxidation and components of important antioxidant enzymes copper and zinc in cardiac tissue after treatment of lead-exposed rats with ascorbic acid, l-methionine or \( \alpha \) tocopherol, chelator CaNa\( \text{2EDTA} \) alone or along with antioxidant \( \alpha \) tocopherol.

**Materials and methods**

*Animals and experimental design.* The experiment was performed using 42 IVRI 2CQ male and female rats sexes weighing about 100 g. Rats were maintained in rat cages in the laboratory animal shed of the division, fed with laboratory animal feed and were provided with water *ad libitum*. Experimental animals were acclimatized to their housing environment one month before the start of the experiment. Six rats received a daily i.p. injection of sterile normal saline solution for a period of 30 days to serve
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as control. The remaining 36 rats were administered with 1 mg Pb²⁺/kg body mass as 0.924% lead acetate (99% pure) solution i.p. for a period of 30 days. The lead and NSS administration were then withdrawn and lead-exposed rats were divided equally into 6 groups. During the next week, rats of each group were either treated with ascorbic acid, l-methionine, or α tocopherol at a daily dose of 100 mg/kg body orally for 7 days, or with CaNa₂EDTA alone at a dose rate of 110 mg/kg-body mass/day intraperitoneally in two divided doses for 4 days, or chelator along with α tocopherol. Those that received no treatment served as positive controls.

Animals of different groups were sacrificed under light anaesthesia one day after the end of the treatment, and the blood was collected from heart. The heart was then quickly excised. Half of the heart was processed immediately for measurement of lipid peroxides level, and the other half was stored at -20 °C for wet digestion, for estimation of lead, copper and zinc content. The blood samples were also wet digested for estimation of lead level (KOLMER et al., 1951).

Biochemical analysis. Lipid peroxides level was estimated through measuring malondialdehyde (MDA) concentration in the cardiac tissue (OHKAWA et al., 1979). This is a useful method for determination of extent of lipid peroxidation, as it is the most abundant aldehyde formed as a by-product during this process (GURER et al., 1998). The values were expressed in nmol of MDA/g of wet tissue using the extinction coefficient of 1.56 x 10⁵ /M/cm (UTLEY et al., 1967).

Analyses of toxic heavy metal and trace elements. The digested samples were analysed for estimation of lead, copper and zinc in the cardiac tissue, and for lead in the blood. The concentration of lead, copper and zinc was measured using absorption spectrophotometer (AAS 4129, Electronic Corporation of India Limited) at 217, 324.7, 239.5 nm wavelength and 6, 5 and 5ma current, respectively, and values were expressed in µg/ml of blood or µg/g of tissue.

Statistical analysis. The data was statistically analysed using one-way analysis of variance to compare the means of different treatment groups (SNEDECOR and COCHRAN, 1994).
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Results

Blood lead concentration in animals administered lead intraperitoneally for 30 days and given no treatment for the next week (0.68 ± 0.04 µg/ml) remained significantly (P<0.05) higher than that of control animals (0.09 ± 0.01 µg/ml). Neither of the treatments with antioxidant or chelator after the end of the lead exposure lowered the blood lead burden to be statistically (P<0.05) comparable to the control animals. The maximum reduction in blood lead level was recorded in rats treated with l-methionine (0.46 ± 0.01µg/ml) followed by EDTA plus α tocopherol (0.64 ± 0.09 µg/ml) and EDTA alone (0.65 ± 0.09 µg/ml). However, treatment with ascorbic acid for 7 days enhanced the blood lead concentration (0.94 ± 0.12 µg/ml) and the mean level was significantly (P<0.05) higher than the rest of the lead-exposed treated groups (Fig. 1).

Table 1 shows the lead burden, lipid peroxides level and copper and zinc concentrations in heart from different therapeutic groups. There was an approximately 13-fold rise in heart lead concentration (5.02 ± 1.06 µg/g) in lead-exposed untreated rats compared to unexposed controls (0.40 ±
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The maximum reduction in tissue lead burden was recorded in animals treated with chelator plus $\alpha$ tocopherol (2.54 ± 0.54 µg/g) followed by chelator alone (3.32 ± 0.72 µg/g), but the tissue concentration in treatment groups remained statistically (P<0.05) comparable to lead exposed untreated group and was significantly higher than that of the unexposed group.

The lipid peroxide level, as determined in terms of nmol of MDA produced/g wet tissue, was non-significantly (P>0.05) higher in rats exposed to lead for 30 days and followed by withdrawal period of next 7 days of treatment duration. The maximum decline (15.4%) in lipid peroxide level in cardiac tissue was recorded in lead-exposed rats treated with chelator plus antioxidant. However, the deteriorative changes in lipids due to oxidative damage were statistically comparable in all groups. Copper and zinc concentration in all treatment groups, including animals given no lead exposure, remained statistically (P>0.05) comparable.

**Discussion**

Lead is a pervasive environmental pollutant that accumulates in almost all tissues (DOYLE and YOUNGER, 1984) and with no beneficial biological
role. Chronic occupational or experimental lead exposure is a risk factor for cardiac diseases and there is a direct relationship between level of exposure and cardiac diseases (BENER et al., 2001). In the present investigation, exposure to lead for a period of 30 days, followed by a period of 7 days of no treatment, led to manifold increases in lead concentration in heart. SINGH et al. (1976) recorded dose-related accumulation of most lead in heart and kidney in newborn rat pups born from the mating of, and nourished by, experimentally lead-poisoned mothers.

The higher concentrations of lead in tissues following occupational or experimental exposure were associated with oxidative damage of DNA, protein and lipids and is has been suggested that lead-induced oxidative stress plays a role in lead-induced toxic effects (MONTEIRO et al., 1985; GURER et al., 1998; PATRA et al., 2001). BENER et al. (2001) recorded a significant association between lead exposure, high blood pressures and risk of heart diseases. Previous animal studies have suggested lead associated oxidative stress in liver and brain (SANDHIR et al., 1994; SANDHIR and GILL 1995; ERCAL et al., 1996). In vitro studies have also indicated the potential of lead in disturbing the pro-oxidant and antioxidant balance (QUINLAN et al., 1988). The resultant oxidative stress, particularly the generation of peroxyl radicals, induces lipid peroxidation, a basic cellular deteriorative change that occurs readily in tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids (CINI et al., 1994). Our earlier studies also revealed increased lipid peroxidation in liver and brain, but not in kidneys following experimental lead intoxication (PATRA et al., 2001). The present finding of a non-significant increase in LPO level in heart might be due to comparatively low oxidizable fatty acids content in heart.

Conventional chelator CaNa2EDTA has long been used for treatment of lead poisoning. The involvement of lead in inducing oxidative stress in liver and brain and the poor chelating ability of this chelator to reduce soft tissue lead burden and its inherent toxicity (CORYSLECHTA et al., 1987) necessitates incorporation of antioxidants for improving therapeutic results (ERCAL et al., 1996). The beneficial role of several vitamins, including ascorbic acid, has been investigated but without being assessed for its potential in ameliorating lead-induced oxidative stress (VIJ et al., 1998, HSU et al., 1998). Vitamin C has earlier been reported as a possible lead chelator...
with a potency similar to that of EDTA (Goyer and Cherion, 1979). In addition, vitamin C is also a low molecular mass antioxidant that interacts directly with oxidizing radicals (Jones et al., 1995). The lipid soluble antioxidant α tocopherol checks lipid peroxidation through limiting the propagation of lipid peroxidation (Buetter, 1993) while methionine serves as a precursor for low molecular mass antioxidant glutathione (Meister, 1981). In the present study none of the antioxidants alone, chelator or chelator plus tocopherol, could significantly (P<0.05) reduce the cardiac tissue lead burden, and lead concentration in the treatment groups remained statistically (P>0.05) comparable to the lead-exposed, untreated group. However, there was maximum reduction (49%) in lead level in groups treated with chelator plus tocopherol followed by chelator alone (34%). This substantiates the earlier finding of reduced mortality in hen supplemented with vitamin E in the feed during concurrent exposure to lead and selenium (Khan et al., 1993).

Copper and zinc are essential components of antioxidant enzymes of the body that play an important role in the prevention of free radical-induced damage to tissues (Evans and Halliwell, 2001). The concentration of such micronutrients in tissue is expected to change due to over utilization of these enzymes, or through regulation of synthesis. Zinc, too, protects the peroxidation of membrane lipids, possibly by displacing bound transition metal ions (Bettiger et al., 1980). In the present study there was no significant (P<0.05) change in copper or zinc concentrations among the different treatment groups. Tissue-specific changes in micronutrient profile have earlier been reported in cattle experimentally exposed to lead (Doyle and Younger, 1984). Our earlier work on young calves showed alterations in trace mineral concentration in blood during the period of lead exposure (Patra et al., 2001). Shaaban et al. (1992) recorded significant correlation between lead and other metals in different tissues. A significant decrease in zinc and copper concentration in heart was recorded in calves administered through the oral route with comparatively higher and toxic doses of lead as compared to the present investigation (Fick et al., 1976). That might have led to reduced absorption of micronutrients from the gastrointestinal tract, besides interaction of lead and trace minerals at tissue level. It is concluded from the study that treatment of lead-exposed rats...
with antioxidant or chelator for a period of only 7 days might not be adequate to reduce the cardiac tissue lead burden.

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SAŽETAK
U pokusu su korištena 42 štakora IVRI 2CQ. Cilj je bio utvrditi učinke antioksidanata askorbinske kiseline, l-metionina i α-tokoferola na nakupljanje olova, peroksidaciju lipidna te koncentraciju bakra i cinka u srčanom tkivu nakon davanja olova. Osim toga, isti pokazatelji analizirani su nakon aplikacije kelata CaNa₂EDTA zasebno, odnosno u kombinaciji s α-tokoferolom. Olovo je aplicirano intraintraperitonealno u obliku 1% otopine olovnoga acetata, u dozi od 1 mg Pb²⁺ na kilogram tjelesne mase tijekom razdoblja od 30 dana. Nakon prestanka davanja olova, štakori (n = 36) su slučajnim odabiru razdijeljeni u šest skupina po šest jedinki u svakoj skupini. Prva skupina bila je izložena samo učincima olova, a pet skupina su osim olova primile navedene antioksidante, kelat ili njegovu kombinaciju s α-tokoferolom. Dodatnih štakora u 7. skupini nisu bili izloženi olovu, antioksidantu ili kelatu te su poslužili kao negativna kontrola. Štakori su žrtvovani u laganoj anesteziji nakon sedmodnevnog dobivanja olova odnosno antioksidanta i kelata. Uzeti su uzorci krvi, a odmah po žrtvovanju izvađeno je srce. Srednja vrijednost koncentracije olova u srčanom tkivu bila je statistički značajno viša u skupini štakora koji su dobivali olovu, čak i nakon prekida sedmodnevnog davanja (5,02 ± 1,06 prema 0,40 ± 0,09 µg/g). Liječenje kelatom u kombinaciji s α-tokoferolom smanjilo je razinu olova u srčanom mišiću, ali je ona i dalje ostala značajno viša u odnosu na negativnu kontrolu. Nije utvrđeno statistički značajno povećanje razine peroksidacije lipid na srčanom tkivu štakora izloženih samo djelovanju olova. Ni jedan antioksidant nije smanjio razinu peroksidacije lipidna, a i srednje koncentracije bakra odnosno cinka nisu se statistički značajno (P>0,05) razlikovale između skupina različito treiranih štakora.

Ključne riječi: olovu, antioksidant, kelat, štakor, srce