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Comparative Effects of Lead on Serum, Liver and Brain High Molecular Weight Alkaline Phosphatase in Rats

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Abstract: The relationship between lead treatment and changes in the concentration of serum, liver and brain high and low molecular weight alkaline phosphatase has been investigated in this manuscript. Results obtained showed that every other day intrapritoneally injection of 39.5 μmol kg^{-1} of lead as ($Pb(CH_3COO)_2.3H_2O$), in male rats for 2 consecutive weeks resulted in decreasing level of liver and brain alkaline phosphatase by 16.7 and 10.9%, respectively, whereas an elevation of serum enzyme activity by 28.4% was seen in comparison to untreated controls ($p<0.05$). Long-term exposure to 13.2 μmol kg^{-1} of this salt, showed a statistically significant reduction in liver and brain levels of alkaline phosphatase by 18.7 and 13.2%, respectively and an increment in serum activity of the enzyme by 37.6% in compared to control group ($p<0.05$). Using gel filtration chromatography technique with sephacryl S_{300} showed that, in comparison to control groups, serum and liver homogenate from lead treated groups had a significant level of high molecular weight alkaline phosphatase, which might be considered as a potential biomarker for lead toxicity.

Key words: Lead, alkaline phosphatase, high molecular weight alkaline phosphatase, liver, brain

INTRODUCTION

The serum of patients with lead (Pb) overload may contain high molecular weight alkaline phosphatase. The presence of this form of alkaline phosphatase (ALP) may be associated with hepatic malignancy (Bhudhisawasdi *et al*., 2004) and thus could be considered as a biomarker in such patients. As a model study, the existence of such isoenzyme has been investigated in sera of Pb treated animals which is the main aim of the present study.

Pb is one of the most ubiquitous heavy metal which has been demonstrated to be neurotoxic (Dabrovaska-Bouta *et al*., 2004). The direct neurotoxic action of Pb include: apoptosis, excitotoxicity, influences on neurotransmitter storage and release process, cerebrovascular endothelial cells and astroglia (Lidsky and Schneider, 2003). Exposure to low level of Pb has been associated with behavioral abnormalities, learning impairment and impaired cognitive function in human and experimental animals (Adonaylo and Oteiza, 1999).

Pb can cause liver damage and may disturb the normal biochemical process in the hepatobiliary system (Sipos *et al*., 2003).

Undesired effects on heme biosynthesis, interference with catecholaminergic and particularly dopaminergic function (NourEddinea *et al*., 2005), lipid peroxidation and free radical mediated cytotoxicity (Mateo *et al*., 2003), altered proliferation and differentiation of neural stem cells (Huang and Schneider, 2004) has been reported following Pb toxicity.

High molecular weight ALP has now been reported in patients with extra- or intra-hepatic cholestasis, malignancy of liver, primary or metastatic carcinoma, Hodgkin's and non-Hodgkin's lymphoma and/or leukemia (Wolf, 1990; Bhudhisawasdi *et al*., 2004) and has been suggested as a tumor marker for liver (Moshtaghie *et al*., 1996) and Colorectal Cancer (Wei *et al*., 1993).

The existence of hepatobiliary dysfunction in those patients with Pb overload (Sipos *et al*., 2003) lead us to investigate and compare the probable occurrence of high molecular weight ALP in sera, liver and brain of rats treated with Pb.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma Chemical Company. Twenty-eight male Wistar rats (approximate weight 200-220 g) were purchased from Pasteur Institute, (Tehran-Iran) and kept in the University Animal House at standard conditions (22-24°C, 40-60% relative humidity and light cycle coinciding with day-light hours) and fed with standard rat food and water ad libitum during the entire experimental period.

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Rats were divided randomly into two groups named: short-term and long-term exposure to Pb, respectively. Each group had its specific control group. In short-term study, control group received every other day intrapritoneally (i.p.) injection of sterile normal saline (0.1 mL) for 2 consecutive weeks, simultaneously treated group was administrated with 39.5 μmol kg^{-1} of Pb ($Pb(CH_3COO)2.3H_2O$) as the same way as controls. Long-term study was carried out using 13.2 μmol kg^{-1} of this salt for a duration of 7 weeks, as described method for the short- term groups.

Rats were then killed by decapitation at the end of their treatment periods. Blood samples were collected and sera were separated from cells by centrifugation and were used for enzyme and protein assay.

Brain and liver tissues were immediately removed, washed with cold (+4°C) saline solution and homogenized (10% w/v) in a buffer solution containing 10 mM tris and 0.25 M sucrose, pH:7.4, at +4°C. The homogenates were then centrifuged at 13000 g for 20 min at +4°C and the resultant supernatants were carefully removed and were used for the enzyme and protein determination (Yazar and Tras, 2001).

Alkaline phosphatase activity was measured at 410 nm and 37°C by the formation of paranitrophenol (pNP) from paranitrophenol phosphate (pNPP) as substrate and 2-amino-2-methyl-1-propanol (AMP) as buffer (Bowers and McComb, 1975).

Protein concentration was determined as described by Bradford, with bovine serum albumin as standard (Bradford, 1976).

Gel filtration chromatography: In order to separate high and low molecular weight isoenzymes of ALP, gel filtration chromatography on sephacryl S_{300} was used. Each sample (0.2 mL) was diluted with equal volume of tris buffer (50 mM, pH 7.4) and was then applied to a column (50×0.9 cm) loaded with sephacryl S_{300} and was then eluted at 10 mL h^{-1} with tris-HCl buffer (50 mM, pH 7.4). Fractions of 1 mL were then collected (Moshtaghie *et al*., 1995). ALP activity and protein concentrations in each fraction were determined according to the methods mentioned earlier (Bowers and McComb, 1975; Bradford, 1976).

Statistical analysis: Analysis of data was accomplished using SPSS (version 11.5) statistical software package. Between-groups comparisons were performed with t-test. All results were presented as Mean±SD and were considered statistically significant at $p<0.05$.

RESULTS

In the first part of this project, short and long term effects of Pb on total serum, liver and brain ALP activities were investigated and the enzyme specific activities were calculated. It was found that administration of Pb in this condition lead to the significant ($p<0.05$) elevation of serum total ALP activity by 28.4% in comparison to normal healthy controls (Table 1A). Significant ($p<0.05$) reduction in the liver and brain total ALP activities by 16.7 and 10.9% was seen when rats were treated with same amount of Pb every other day for two weeks (Table 1A).

Long term study was performed by injection of Pb every other day for seven weeks. Measurement of total serum, liver and brain ALP activities was performed. Results obtained are presented in (Table 1B). Significant ($p<0.05$) elevation of 37.6% in total serum ALP and significant ($p<0.05$) and reduction of 18.7 and 13.2% in liver and brain ALP were seen.

Comparing the data obtained from short and long term effects of Pb on the activity of the enzyme in serum, liver and brain showed that changes in the enzyme activity was dose and time dependent processes. It should be emphasized that the more Pb administration for long term the higher the effects on enzyme activity.

Gel filtration chromatography technique: Second series of experiments were established to separate high and low molecular weight ALP from sera, liver and/or brain homogenate of both treated and untreated animals. To do this, gel filtration chromatography technique was used. Aliquots (0.2 mL) of either serum, liver and/or brain homogenates were diluted with 0.2 mL of buffer and loaded to the top of the column. The column was then eluted as mentioned in method section. Fractionation of the serum from Pb treated and untreated control

Table 1: Effect of Pb on the activity of serum, liver and brain ALP activity. Rats were injected with Pb as ($Pb(CH_3COO)_2.3H_2O$) every other day for two weeks (A) and for seven weeks (B). Animal were killed and ALP activity was determined

	ALP (IU mg^{-1} tissue protein)		
	Serum	Liver	Brain
A			
Control	1.94±0.10	3.42±0.26	2.02±0.12
Treated	2.49±0.18*	2.85±0.13*	1.80±0.07*
B			
Control	2.10±0.10	3.16±0.26	2.27±0.13
Treated	2.89±0.12*	2.57±0.17*	1.97±0.09*

* Indicates statistically significant difference of ALP activity between Pb treated animals and their controls ($p<0.05$). Data are presented as Mean±SD

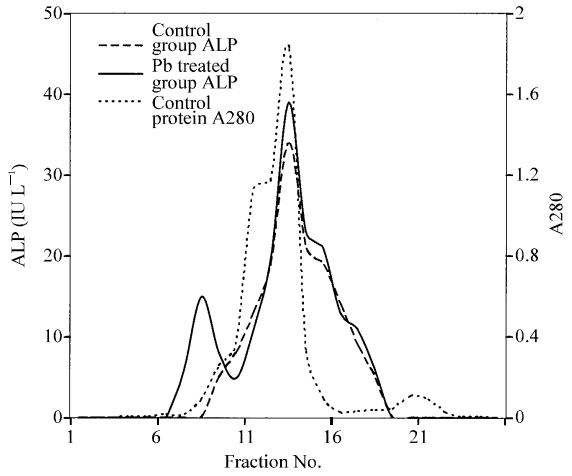

Fig. 1: The elution profile of serum of control and Pb treated groups

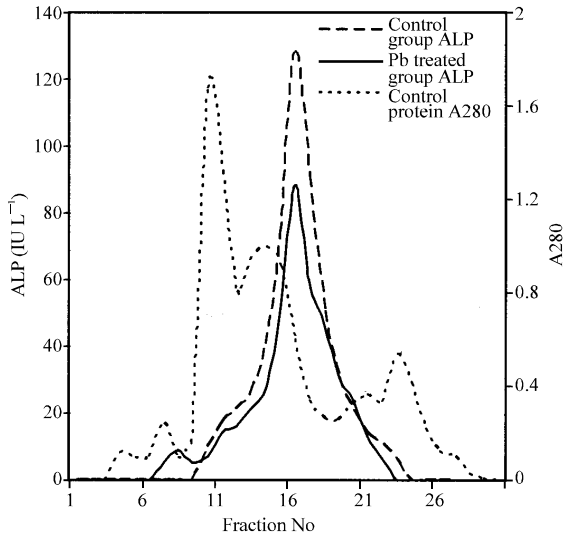

Fig. 2: The elution profile of liver of control and Pb treated groups

animals showed that the elevation in the serum total ALP activity was mostly related to the high molecular weight ALP (Fig. 1).

When liver homogenate from both control and Pb treated rat was chromatographed, a significant reduction of 31.25% in the activity of low molecular weight ALP and a significant ($p<0.05$) elevation in high molecular weight alkaline phosphatase (Fig. 2) was found. Interestingly, a decrease in total liver homogenate ALP activity and concomitant elevation of high molecular ALP was seen, which were both elevation statistically significant.

Figure 3 shows a significant ($p<0.05$) reduction in low molecular weight by 28.1% in brain ALP following Al treatment, but no changes was observed in the level of

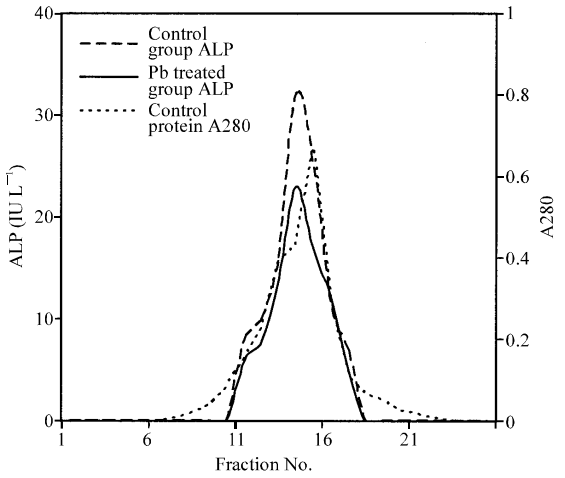

Fig. 3: The elution profile of brain of control and Pb treated groups

high molecular weight ALP when compared with control values.

DISCUSSION

Measurement of the activities of ALP isoenzymes has been used for the identification and monitoring of such diseases associated with the isoenzymes. Biliary ALP, or high molecular weight ALP has been found in the serum of patient with biliary obstruction and metastatic liver cancer (Bhudhisawasdi *et al*., 2004). Previous study from this laboratory showed that high molecular weight ALP could be considered as a tumor marker for liver cancer (Moshtaghie *et al*., 1996). This isoenzyme could be regulated by steroid hormones (Moshtaghie *et al*., 1995) and also affected by other elements such as aluminium (Moshtaghie *et al*., 2006). However, up to our knowledge no data has presented in the literature concerning Pb toxicity and the induction of high molecular weight ALP in the serum of patients with Pb overload. Results presented in this study, revealed the relationship between Pb administration and changes in the sera, liver and/or brain high molecular weight ALP.

Data present in this manuscript showed that following short and long terms of Pb administration to rat increased total serum ALP activity significantly ($p<0.05$), whereas, liver and brain total ALP activities decreased (Table 1). These changes were a dose and time dependent processes. Similar results have been reported by others. They have demonstrate that other enzymes such as: acid phosphatase, ATPase, acetylcholine esterase were decreased in brain (Antonio *et al*., 2003; Antonio and Leret, 2000).

Decreased in the activity of ALP in liver and brain may be due to inhibiting the synthesis of ALP activity by the replacement of zinc substitution, or indirectly decreasing zinc availability for ALP synthesis (Antonio *et al*., 2003).

When serum total ALP activity elevated for Pb treated animals was fractionated, it showed that elevation of serum total ALP activity was mostly due to the high molecular weight ALP in Pb treated group in comparison to control group (Fig. 1). Elevated high molecular weight ALP was also found in liver homogenate in the Pb exposed group (Fig. 2), but there was no high molecular weight ALP in brain homogenate (Fig. 3). The elevated high molecular weight ALP in serum may be originated either from the liver and/or other tissues producing this enzyme. This may also be due to either damage of bile duct and/or synthesis of new molecules of high molecular weight ALP. Alternatively association of low molecular weight ALP with other enzymes including 5'-nucleutidase, γ-glutamyltranspeptidase and nucleotide-pyrophosphatase, could be resulted in the formation of high molecular weight ALP (Wulkan and Leijense, 1986; Remaley and Wilding, 1989).

Comparing data obtained from liver and brain (Fig. 2 and 3), showed that although low molecular weight ALP significantly decreased in the brain of Pb treated animals, no indication was seen in the production of high molecular weight ALP.

It may be concluded that Pb could make pathophysiological damage to liver tissue, particularly, bile ducts leading to the production and secretion of high molecular weight ALP. However, the appearance of this isoenzyme in the sera could be considered as a suitable tools in the diagnosis of Pb toxicity. At this point we try to develop a reliable and fast method for routine measurement and diagnosis of high molecular weight ALP in medical laboratories.

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