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

A.A. Moshtaghie, M. Ani and S.M. Mirhashemi

The relationship between aluminium 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 intraperitoneally injection of 186 μmol kg⁻¹ of aluminium (AlCl₃·6H₂O) in male rats for 2 weeks resulted in decreasing the level of liver and brain alkaline phosphatase by 14.9 and 9.9%, respectively, whereas an elevation of serum levels of this enzyme by 21.1% was seen in comparison to untreated controls (p<0.05). Long-term exposure to 74.5 μmol kg⁻¹ of this salt, showed a statistically significant reduction in liver and brain levels of alkaline phosphatase by 15.8 and 12.3%, respectively and an increment in serum activity of the enzyme by 30.9% in compared to control group (p<0.05). Using gel filtration chromatography technique with sephacryl S₃₀₀ showed that, in comparison to control groups, serum and liver homogenate from aluminium treated groups had a significant level of high molecular weight alkaline phosphatase, which might be considered as a potential biomarker for aluminium toxicity.

Key words: Aluminium, alkaline phosphatase, high molecular weight alkaline phosphatase, liver, brain
INTRODUCTION

The serum of patients with Aluminium (Al) 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 Al treated animals which is the main aim of the present study.

Al is a ubiquitous element in the environment and has been demonstrated to be neurotoxic, especially in individuals with renal failure (Klein, 2005). The well described long-term effects of sustained exposure to Al in patients with end stage renal failure is the result of uptake and storage of Al leading to cellular toxicity (Fanswick et al., 2005). This element is present in many manufactured foods and medicines and is also added to drinking water for purification purposes (El-Demerdash, 2004).

This element, for a long time, has been considered as an indifferent element from a toxicological point of view. In recent years, however, Al has been implicated in the pathogenesis of several clinical disorders, such as dialysis dementia, dialysis osteodystrophy and hypochromic microcytic anemia (Osinska et al., 2004). Al enters blood circulation via dialysis fluid and binds to serum transferrin (Moshaghie et al., 1992) a major iron carrier protein. The uptake and accumulation of Al by hepatocytes has already been reported (Moshaghie, 1994). This toxic metal affects many tissues, particularly brain, liver, bone, kidney, blood (Oteiza et al., 1993), etc. Impairments of glucose utilization, agonist-stimulated insulin phosphate accumulation, free radical- mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation has been reported following Al toxicity (Strong et al., 1996).

High molecular weight ALP was first reported by Fritsche and Adam-Park, in 1972. This isoenzyme 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; Bhudhisawasli et al., 2004) and has been suggested as a tumor marker for liver (Moshaghie et al., 1996) and Colorectal Cancer (Wei et al., 1993).

The existence of cholestasis in those patients with Al Overload (Gonzalez et al., 2004) lead us to investigate and compare the probable occurrence of high molecular weight ALP in sera, liver and brain of rats treated with Al.

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 University Animal House at standard conditions (22-24°C, 40-60% relative humidity and light cycle coinciding with daylight h) and fed with standard rat food and water ad libitum during the entire experimental period.

Rats were divided randomly into two groups named: short-term and long-term exposure to Al, respectively. Each group had its specific control group. In short-term study, control group received every other day intraperitoneally (i.p.) injection of sterile normal saline (0.1 mL) for 2 weeks, simultaneously treated group was administrated with 186 μmol kg-1 of Al (AlCl3·6H2O) as the same way as controls. Long-term study was carried out using 74.5 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 centrifuged at 13000 g for 20 min at +4°C and the resultant supernatants were carefully removed and 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 (pNP) as substrate and 2-Amino-2-Methyl-1-Propanol (AMP) as buffer (Bomers 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 S300 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 (50×9 cm) loaded with sephacryl S300 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 (Moshaghie et al., 1995). ALP activity and protein concentrations in each fraction were determined according to the methods mentioned earlier (Bomers 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 present study, short and long term effects of Al on total serum, liver and brain ALP activities were investigated and the enzyme specific activities were calculated. It was found that administration of Al in this condition lead to the significant (p<0.05) elevation of serum total ALP activity by 21% in comparison to normal healthy controls (Table 1). Significant (p<0.05) reduction in the liver and brain total ALP activities by 14.9 and 9.9% was seen when rats were treated with same amount of Al every other day for two weeks (Table 1).

Long term study was performed by injection of Al every other day for seven weeks before the measurement of serum, liver and brain total ALP activity. Results obtained showed significant (p<0.05) elevation of 30.9% in total serum ALP and significant (p<0.05) reduction of 15.8 and 12.3% in liver and brain ALP were seen in Table 1.

Comparing the data obtained from short and long term studies effect of Al on the activity of the enzyme in serum, liver and brain was both dose and time dependent processes. It however should emphasis that Al administration in long term showed much higher 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 as mentioned earlier. 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 and fractions were collected.

Table 1: Effect of Al on the activity of serum, liver and brain ALP activity

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Liver</th>
<th>Brain</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>1.94±0.01</td>
<td>3.42±0.26</td>
<td>2.02±0.12</td>
</tr>
<tr>
<td>Treated</td>
<td>2.35±0.13*</td>
<td>2.91±0.20*</td>
<td>1.82±0.11*</td>
</tr>
</tbody>
</table>

Rats were injected with Al as (AlCl3.6H2O) every other day for two weeks (A) and for seven weeks (B). Animal were killed and ALP activity was determined. *indicates statistically significant difference of ALP activity between Al treated animals and their controls (p<0.05). Data are presented as Mean±SD.

Fractionation of the serum obtained from aluminium treated and untreated control 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 aluminium treated rat was chromatographed, a significant reduction of 28.9% 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 33.3% in brain ALP following Al treatment, but no changes was observed in the level of 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 (Bhadhiaswasi et al., 2004). Previous study from this laboratory showed that high molecular weight ALP could be considered as a tumor marker for liver cancer (Mohijie et al., 1996). This isoenzyme could be regulated by steroid hormones (Mohijie et al., 1995). However to our knowledge no data has presented in the literature concerning AI toxicity and the induction of high molecular weight ALP in the serum of patients with AI overload. Results presented in this study, revealed the relationship between AI administration and changes in the sera, liver and/or brain high molecular weight ALP.

Present findings showed that following short and long terms of AI administration to rat total ALP activity in serum increased significantly (p<0.05), whereas, liver and brain total ALP activities decreased (Table 1). These changes were a dose and time dependent processes. The present data are consistent with previous findings by El-Demerdash (El-Demerdash, 2004), that other enzymes such as aspartate aminotransferase, alanin aminotransferase, were significantly decreased in the liver, while the activities of these enzymes increased in serum following AI treatment. Decreased in the activity of ALP in liver and brain may be due to binding of Al to DNA and RNA and inhibiting synthesis of low molecular fraction of the enzyme (Ochmanski and Barbas, 2000).

Yosef (2004) also showed that AI administration to rat reduced total activity of aspartate aminotransferase, alanin aminotransferase and alkaline phosphatase. This finding was previously reported by our laboratory (Mohijie et al., 1996). Chronic AI exposure leads to oxidative stress, cholestasis and impairment of hepatic handling of organic anions by decreasing both sinusoidal uptake and canalicular excretion (Gonzalez et al., 2004).

When serum total ALP activity was fractionated, it showed that elevation of serum total ALP activity was mostly due to the high molecular weight ALP in Al treated group in comparison to control group (Fig. 1). Elevated high molecular weight ALP was also found in liver homogenate in the AI 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 S-nucleotidase, γ-glutamyltranspeptidase and nucleotide-pyrophosphatase, could be resulted in the formation of high molecular weight ALP (Wulkan and Leijen, 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 Al treated animals, no indication was seen in the production of high molecular weight ALP.

It may be concluded that AI could make pathophysiological damage to liver tissue, particularly, bile ducts leading to the production and secretions of high molecular ALP. However, the appearance of this isoenzyme in the sera could be considered as a suitable tool in the diagnosis of AI toxicity which is frequently seen in renal patients maintained on haemodialysis. 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.
REFERENCES


