Changes in the Level of Mitochondrial and Cytosolic Aspartate Aminotransferase Activities in Aluminium Intoxified Rat

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ABSTRACT

The activity of aspartate aminotransferase (AST) in human serum has been widely determined as a diagnostic aid in liver disease. In this study, the effect of aluminium on AST isoenzymes in relation to aluminium intoxified patients has been investigated. Using gel filtration chromatography technique with Sephacryl S-300, mitochondrial aminotransferase (m-AST) and cytosolic aminotransferase (c-AST) fractions were separated from rat serum and liver homogenate. The c-AST fraction was eluted with higher mobility than m-AST iso-enzyme. Daily administration of aluminium (1 and 5 mg/kg body weight) for 30 days increased total serum activity of AST by 19% and 72%, respectively. Daily administration of aluminium (10 and 20 mg/kg body weight) for 30 days was also studied. The percentage of elevations was 114% and 86% in comparison to the controls. Following aluminium administration for 45 days, the enzyme activity was elevated to 20% and 60% in comparison to the controls, and administration for 60 days resulted elevation of 35% and 79%. The serum enzyme activity was mostly due to the mitochondrial fraction of AST that was a time and dose dependent. *Iran. Biomed. J. 7 (4): 167-171, 2003*

INTRODUCTION

The importance of aspartate aminotransferase (AST) in the diagnosis of a number of disorders of liver, heart, etc., has been well documented. Recent evidences suggest the existence of two isoenzymes for aspartate aminotransferase namely mitochondrial aminotransferase (m-AST) and cytosolic aminotransferase (c-AST) [1]. Using a number of biochemical techniques including electrophoresis, column chromatography and immunochemical, the two isoenzymes have been separated, and characterized by many investigators [2-4].

The patients who are undergoing regular hemo-dialysis for chronic renal failure suffer from a number of disorders including hypochromic microcytic anemia [5], dialysis osteodystrophy [6], neurological disorders including dialysis dementia [7] and Alzheimer's disease [8]. It is well documented that aluminium is a contaminating agent of dialysis solution [9]. It transfers across dialysis membrane and enters into the blood circulation where it binds to the serum proteins, particularly transferrin [10]. Aluminium- transferrin (Al-tf) complex is taken up by cells through transferrin receptors [11]. In the cells, the majority of aluminium binds to the nuclei, mitochondrial and cytosolic compartments [12]. The accumulation of aluminium in the cell organelles could therefore disturb many biochemical processes including carbohydrate [13], lipid [14] and iron metabolism [15]. Up to now, no evidence available in the literature reporting changes in the serum m-AST and c-AST following aluminium accumulation in patients with aluminium overload who are undergoing hemodialysis. Therefore, we have established an animal model study to investigate the effect of aluminium on the serum m-AST and c-AST activities in short and long terms. The major goal of this project was to determine a probable serum marker for aluminium toxicity in relation to the aluminium overload in hemodialyzed patients.

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MATERIALS AND METHODS

All chemicals used in this project were of analytical grade and were purchased from Sigma Chemical Company unless otherwise stated.

Animals. Male Wistar rats (150-200 g) were purchased from the Pasteur Institute of Iran (Tehran) and were fed with water and commercial Purina Chow ad libitum at standard conditions regarding light and temperature. Animals were studied until they reached within the range of 220-250 g body weight (BW). In each series of experiments, 4 aluminium treated and 4 control rats were used. Each rat received aluminium (1 to 20 mg/kg BW) as aluminium chloride in 0.2 ml saline. Controls were injected only 0.2 ml saline. At the end of injection time (1 to 60 days), rats were killed by decapitation. The blood was collected in pre-washed plastic tubes for determination of the total serum AST activity and also for the separation of serum AST iso-enzymes by gel-filtration chromatography.

Preparation of rat liver mitochondria. Livers of the decapitated animals were removed and put on ice-cooled water bath at 4°C. The livers were trimmed, rinsed, blotted dried, weighted and placed into 5 volumes of ice-cold 0.25 M sucrose. Subcellular fractions were prepared using ultracentrifugation technique by the method reported elsewhere [12].

Gel filtration chromatography. This technique was used to separate mitochondrial and cytosolic AST from serum sample and also from liver homogenate. Column (50 × 0.9 cm) was packed with Sephacryl S-300 and equilibrated with Tris/HCl buffer (10 mM, pH 7.4). Two ml of either serum or mitochondrial fraction was diluted with 2 ml buffer and loaded on the column and then column was eluted with the same buffer [16]. The AST activity in the fractions was determined in each individual fraction according to the method reported elsewhere [17]. For statistical analysis student's *t*-test was used at $P<0.05$. Aluminium determination was performed using flameless atomic absorption spectrophotometry technique with grafite furnace [18].

RESULTS

Initially, the level of serum aluminium was measured in animals that received various amounts of aluminium (2 to 20 mg/kg BW) as aluminium chloride for 1 to 20 days. Controls received only saline (Table 1). Significant elevation of aluminium was found in the serum following aluminium administration. The elevations were dose and time dependent.

In the second series of experiments, short and long term effects of aluminium on total serum AST activity were investigated. To achieve this, aluminium as aluminium chloride (1, 5, 10 and 20 mg/kg BW) was injected i.p. daily for 30, 45 and 60 days and then the serum AST activity was determined (Table 2). Daily aluminium administration (1 and/or 5 mg/kg BW) for 30 days elevated AST activity by 19% and 72%, respectively (Table 2). At the same time, when the effect of 10 and 20 mg/kg of aluminium was studied, the percentages of elevation were 114% and 86 % in comparison to the controls (Table 2).

Table 1. Aluminium determination in rat serum. Rats were injected daily with various amounts of aluminium (1 to 60 days). Animals were killed by decapitation at the end of treatment. Blood were collected, sera were separated and serum aluminium concentration was determined. Each number is Mean ± SE of 4 different determinations. For more details see Materials and Methods.

Aluminium dose (mg/kg)	Injection times (Days)	Serum aluminium (µg/l)	
		Control	**Treatment**
2	15	1.1 ± 0.050	22.5 ± 3.0
2	30	1.5 ± 0.007	37.5 ± 6.6
2	60	1.2 ± 0.060	54.1 ± 6.3
5	15	1.5 ± 0.050	36.7 ± 2.8
5	30	1.4 ± 0.040	86.0 ± 5.2
10	15	1.0 ± 0.050	84.0 ± 7.3
10	30	1.5 ± 0.070	159.1 ± 10.1
20	1	1.5 ± 0.070	12.1 ± 3.2

Table 2. Effect of aluminium on total serum aspartate aminotransferase activity. Rats were injected daily with various amounts of aluminium and were decapitated at the indicated times. Sera were collected and total AST activity was determined in each sample. Each number is mean ± SE of 4 different determinations. For more details see Materials and Methods.

Aluminium injected (mg/kg BW)	Aspartate Transaminase activity (IU/L)		
	30 days	**45 days**	**60 days**
None	87.6 ± 9.7	91.5 ± 12.2	91.0 ± 12.5
1	*105.0 ± 2.2	110.8 ± 8.3	*122.5 ± 9.3
5	*150.7 ± 10.6	*158.2 ± 6.5	*163.5 ± 8.2
10	*188.7 ± 10.1	*200.6 ± 9.6	*240.1 ± 10.2
20	*162.3 ± 8.7	*180.1 ± 6.8	*190.2 ± 12.1

*Indicates significant changes at $P<0.05$.

Following daily injection for 45 and 60 days, the effects of 1 and 5 mg/kg BW of aluminium on total serum AST activity were also studied. Aluminium administration (1 and 5 mg) for 45 days elevated the serum enzyme activity by 20% and 60%, respectively. Following 60 days of aluminium administration, the total serum AST activity was increased by 35 and 79%, respectively (Table 2). Daily effects of 10 and 20 mg/kg BW of aluminium on total serum AST activity were also studied for 45 and 60 days. Aluminium injection (10 mg) elevated total serum AST activity by 120% and 163% and 20 mg/kg BW of aluminium increased total serum AST activity by 197% and 108%, respectively (Table 2).

The effect of aluminium on serum mitochondial and cytosolic AST activities. In order to find out which fraction of serum AST isoenzymes was mostly affected by aluminium, sera from controls and from aluminium treated animals were loaded on the column and eluted with Tris/HCl buffer. Total serum AST activity was then determined in 40 eluted fractions, each of them contained two ml of the eluted solution. When data were plotted
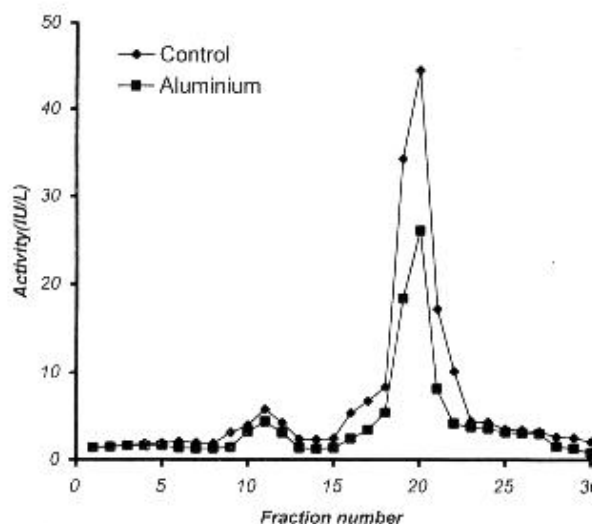

Fig. 2. Rat liver AST mitochondrial activity profile from untreated aluminium. Healthy rat was killed by decapitation. Liver was immediately removed and mitochondria was prepared and loaded on the column and eluted according to the Materials and Methods. The AST activity was then measured in eluted fraction.
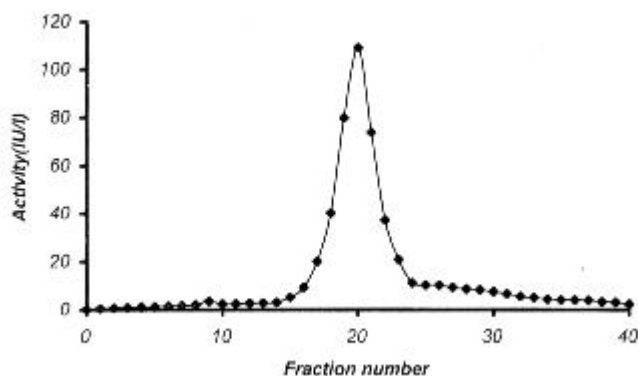
(enzyme activity versus fraction number), two major peaks were obtained. The first peak corresponded to the AST with less enzyme activity and the second one had higher AST activity. When sera from aluminium treated animals were loaded on the same column and eluted the same way as controls, the aluminium administration elevated the AST activity of the second peak by 76 % in comparison to the controls. (Fig. 1). To identify these two peaks, mitochondrial fractions of rat liver were prepared as mentioned in Materials and Methods and further washed with 0.25 M sucrose. The purity was checked by the method reported previously [12]. Two ml of completely homogenized mitochondria in 0.1 ml Triton-X100 was diluted with Tris/HCl buffer (100 mM, pH 7.4) and loaded to the column. The column was eluted with the same buffer and AST activity of rat liver

Fig. 1. AST iso-enzyme activity profiles from serum control and aluminium treated animals. Sera from a control and aluminium treated rats (5 mg aluminium/kg BW) for 60 days were loaded on the column and then were eluted. The AST activity was measured in each individual fraction and the profiles were obtained. For more information see Materials and Methods.

mitochondria in each fraction was determined and compared with serum m-AST profile (Fig. 2).

DISCUSSION

Our data show that aluminium administration to rat as a model study leads to the elevation of serum aluminium. This elevation depends on the amount of aluminium administration and also duration of aluminium injection. Sampson *et al.* [19] found that the serum aluminium level in renal patients maintained on hemodialysis was high that correlates with the intake dose and duration of aluminium consumption $Al(OH)_3$ in chronic renal failure. Our data also indicated that aluminium administration leads to the serum elevation of total AST activity, suggesting probable liver damage in aluminium intoxified rat [17]. Other observations show elevation of serum aluminium in dialysis patients with chronic liver disease [20].

Changes in the activity of serum aspartate aminotransferase isoenzymes in patients with acute myocardial infarction have been reported by Panteghini *et al.* [21]. They also indicated that serum cytosolic and mitochondrial AST activity increased after onset of chest pain and m-AST disappeared from the serum slower than c-AST. These two AST isoenzymes already isolated from serum were characterized [22]. Our findings indicate that aluminium administration leads to the serum elevation of total AST activity and this elevation is mostly related to m-AST (Fig. 1). The elevation may also be due to the release of AST from liver mitochondria, suggesting a damage to mitochondrial function, or the stimulation of m-AST synthesis following aluminium influx to the cells. There are some other factors that may influence the activity of c-AST and m-AST. Abnormal increase of calcium ions in ischemic retinal cells may cause an influx of calcium ions into the mitochondria, with subsequent reduction in m-AST showing that calcium involves in the degradation of m-AST [23].

The interference of aluminium with calcium ion metabolism has been already well documented [24]. Aluminium interferes with calcium absorption and causes bone disease named as dialysis osteodystrophy [25]. The elevation of m-AST may indicate that aluminium interferes with calcium uptake by mitochondria that lead to the elevation of m-AST activity. Our data shows m-AST may provide a good tool and also provides more diagnostic information from other traditional liver enzymes commonly used in aluminium intoxified patients with liver disease [26]. Furthermore, the elevated serum m-AST may suggest damages to other mitochondrial functions including disturbances of heme synthesis reported in aluminium intoxified patients [20, 27].

More studies are needed to elucidate the exact mechanism by which aluminium interferes with mitochondrial function and the use of m-AST as a probable marker for aluminium toxicity in renal patients with aluminium overload. Finally, the aluminium contents of dialysis fluid, water supply and also sera of hemodialyzed patients should be determined regularly in order to prevent mitochondrial damage in these patients.

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