اثرات آلومینیوم بر غلظت آنزیمهای ALT, AST, LDH

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چکیده :

مسمومیت با آلومینیوم در دهه‌های اخیر یکی از گزارش‌های قابل مسلمی است که مراکز دیالیز دنیا در مقالات علمی اشاره گردیده‌است. این عنصر سمی از یک طرف در مایع دیالیز و از طرف دیگر به صورت ترکیبات کاهش‌دهنده فسفات در بیماران کلیوی که از سیستم دیالیز استفاده می‌کنند وارد خون شده و عوار ض مختلفی در آنها ایجاد می‌کند. در پروژه حاضر پس از راه‌اندازی چگونگی انجام آلومینیم بوسیله دستگاه *Flameless atomic absorption* اثرات این عنصر سمی را بر روی فونکسیون کبد با اندازه‌گیری پارامترهای مربوط مورد مطالعه قرار دادیم.

نتایج حاصل نشان می‌دهد تزریق *1* , *5* و *10* میلی‌گرم آلومینیوم به ازاء هر کیلوگرم وزن بدن در مدت ۳۰ روز سبب افزایش قابل توجه آنزیمهای *AST,LDH* و *ALT* و همچنین , پروتئین‌های سرم می‌گردد، که میزان افزایش برای *LDH* به ترتیب (۸، ۳۰ و ۷۰ درصد)، *AST* به ترتیب (۲۰، ۶۵ و ۱۱۴ درصد) و *ALT* (۱۷۴، ۲۸۱، ۳۴۶ درصد) می‌گردد.

تزریق داخل صفاقی آلومینیم به میزان ۱ و ۵ و ۱۰ میلی‌گرم در کیلوگرم وزن بدن به مدت ۶۰ روز تغییرات چشمگیری‌تری بر میزان پارامترهای بالا گذاشته است.

با توجه به تغییرات غلظت هریک از پارامترهای *LDH* و *AST* و *ALT* و بیلیروبین در سرم خون نشان می‌دهد که آلومینیم می‌تواند بر فونکسیون کبد تأثیر گذاشته و در راههای متابولیسمی مواد مختلف تأثیر بگذارد.

کلید واژه‌ها: ۱ـ مسمومیت با آلومینیوم ۲ـ سرم ۳ـ کبد ۴ـ موش صحرائی

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ALUMINIUM TOXICITY AND CHANGES IN SERUM PARAMETERS RELATED TO LIVER FUNCTION IN RATS

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ABSTRACT

The effect of aluminium on serum parameters related to liver function was investigated over different period of times.

Serum lactate Dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanin aminotransferase (ALT) and total protein were chosen as indexes for liver function. Daily intraperitoneal administration of 185 μmoles/kg aluminium for 35 days elevated serum aluminium concentration from 10±0.5 to 668±81 μg/L.

Short term administration of 740 μmoles/kg of aluminium daily for 10 days elevated LDH, AST, ALT and bilirubin concentrations by 27%, 18%, 62% and 60% respectively in comparison to controls. Daily administration of 35, 185 and 370 μmoles/kg of aluminium for 60 days elevated serum levels of LDH (33%, 47% and 69%), AST (29%, 34% and 70%), ALT (31%, 39% and 77%) and bilirubin (26% , 36% and 41%) respectively.

Protein concentration was elevated significantly. Aluminium administration might disturb liver function by interferring with biochemical pathways.

The interference of aluminium with parameters related to liver function has been discussed here.

Key Words: *1) Aluminium toxicity* *2) Serum* *3) Liver* *4) Rat*

INTRODUCTION

Aluminium is present in very small amounts in living organism but there is now available sufficient unequivocal evidence to indicate that aluminium is a toxic agent in patients with chronic renalt failure main -tained on regular hemodialysis. The initial

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description of potential aluminium toxicity in renal failure patients relates to description of dialysis encephalopathysyndrome in 1972 and subsequently in 1976[1,2]. Alfray et al in 1976 showed that aluminium could readily cross dialysis membranes and lead to the elevation of serum hyperaluminium in patients on regular hemodialysis. Aluminium from either dialysis fluid[3] or aluminium phosphate binders[4]enters blood circulation and binds to serum transferrin[5].

In addition to neurological disorders aluminium has been also reported to produce dialysis osteomalacia[6] and hypochromic microcytic anemia[7]. In addition to above mentioned disease London et al recently noted that, in a group of patients with end stage renal disease tissue aluminium levels correlated directly with left ventricular mass and inversely with the velocity of circumferential fiber shorten -ing, implicating aluminium overload as a potential cause of reduced cardiac function [8]. Diabetics have an increased risk of developing renal insufficiency, as well as congestive heart failure independent of coronary atherosclerotic or hypertensive heart disease.

Accumulation of aluminium in tissues of patients with diabetics has been recently reported. aluminium has been also reported to make damage to lipid metabolism and decreased triglyceride levels in rats[10]. Since liver is the major site of lipid metabolism, any damage to this organ might also disturb other biochemical pathways.

The present project was undertaken to investigate the short and long term effects of aluminium on serum parameter levels related to liver function. To follow this aim serum LDH, AST, ALT, bilirubin and protein concentrations were chosen.

MATERIALS AND METHODS

Male Wistar rats (150-200grams) were purchased from Pasteur Institute (Tehran, Iran) and were fed with commercial purina chow at standard conditions regarding light and temperature. Studies were carried out with animals that weighed within the range of 220-250 grams.

In each series of experiments 5 alumi-nium treated and 5 control rats were used. Indicated doses of aluminium was injected in 0.2 ml of saline. Controls were injected with 0.1 ml of saline. The onset and duration of each injection series are given in the text. At the end of injection times, rats were killed by decapitation and blood samples were collected in pre-washed plastic tubes. Sera were separated from blood cells by centrifugation at 300 rpm and transferred into pre-washed tubes for aluminium and other serum parameter determinations.

To estimates the aluminium cotent of serum a flameless atomic absorption

spectrophotometers (Model Perkin Elmer zeeman-3030) fitted with HGA-6000 electro thermal furance was used. Serum samples were diluted with equal volume of 10% HNO3 and aluminium was determined in serum as described by Rahman et al[11].

Serum LDH, AST and ALT levels were determined by using spectrophotometric methods reported by Henry et al[12].

Serum protein was determined by Lowry method [13] and total serum bilirubin concentration was determined by the method of Malloy and Evelyn [14].

All chemicals were reagent grade and purchased from Sigma Chemical Company (Germany).

Throughout this project, all glass ware were soaked overnight in 10% HNO3, and then throughly rinsed with distilled and deionized water so as to minimize metal contaminations.

Plastic ware were pre-washed with 10mm EDTA followed by three washes each of distilled and deionized water.

RESULTS

In the first instance aluminium(185 μmoles/kg BW) as aluminium choloride in saline was administered (i.p) daily for 35 days. At the end of injection times animals were killed and serum aluminium level was determined. Control groups were injected daily with saline (see methods). The aluminium level was significantly elevated in comparison to controls. Serum aluminium level follwing 10,15 and 35 days of aluminium administration was (185±21),(237±37) and (668±81) μg/l respectively. Whereas in control it was 10±0.5 μg/l.

In the second series of experiments, the effects of varying amounts of aluminium on serum LDH, AST and ALT concentrations were studied.Rats were injected (ip) with 740 μmoles/kg body weight with aluminium as aluminium choloride in saline daily for 10 days. The results obtained show that serum levels of LDH,AST and ALT were elevated by 26%,18% and 62% respectively in comparison to un-injected aluminium (table1).

The effects of 35, 185 and 370 μmolos aluminium/kg BW on the same parameters were atudied after daily administration of aluminium for 30 days. Results are presented in (table 2). It shows that LDH level was elevated by 3%, 7% and 30% following 35, 185 and 370 μmoles/kg BW aluminium administration. Daily administartion of 35, 185 and 370 μmoles/kg BW of aluminium for 3 day elevated ASTactivity by 10% , 37% and 63% and at the same time ALT activity was increased by 75%, 116% and 133% respectively in coparison to un-injected aluminium controls (table 2).

Table 1 :Study of the effect of aluminium on serum LDH, AST and ALT in male rat for 10 days

Aluminium dose	Serum Enzyme Specific activities		
μmole/kg	LDH	AST	ALT
None	19.1±1.8	2.3±0.12	1.3±0.27
740	24.1±0.6*	2.7±0.6*	2.1±0.3*
	(26%)	(18%)	(62%)

Table 1.Rats were injected intraperitoneally, daily for 10 days with aluminium as AlCl3 in O.2 ml of Nacl (0.9/100). Controls were received dialy 0.2ml of saline. Animals were killed and blood samples were collected as mentioned in Materials and Methods. Serum LDH, ASTand ALT levels were deter-mined. Each figure is the Mean± SD of five separate experiments. Enzyme activities were calculated as International unilt per lit (Iu/l) and expresed as specific activities. Statistically significant differences from the controls, where $P<0.05$.(*)

Table 2 :Study of the effect of aluminium on serum LDH, AST and ALT activities in male rat for 30 days

Aluminium dose	Enzyme Specific Activities		
μ/kg BW	LDH	AST	ALT
None	22.1±9.4	1.9±0.3	1.2±0.2
35	22.9±2.05	2.1±0.2	2.1±0.7*
	(+3%)	(+10)	(+75%)
185	23.7±6.7	2.6±0.1*	2.6±1.5*
	(7%)	(+37%)	(+116%)
370	28.9±4.5*	3.1±0.5*	2.8±0.5*
	(+30%)	(63%)	(+133%)

Table 2.Rats were administered with aluminium daily for 30 days. Controls and aluminium injected animals were treated as described in table 1.

Long term effects of aluminium on the same parameters were studied next. To do this 60 days of aluminium administration was chosen. Daily administration of 35, 185 and 370 μmoles aluminium/kg BW elevated LDH by 33%, 47% and 69%, AST activity

was elevated by 29%, 34% and 70% respectively. ALT level was elevated by 31%, 39% and 77% respectively (table3).

Table 3 :Study of the effect of aluminium on serum LDH, AST and ALT activities in male rat over 60 days

Aluminium dose µmole/kg BW	Serum Enzyme Specific activities LDH	AST	ALT
None	22.4±1.5	1.7±0.2	1.3±0.7
35	29.7±2.8* (+33%)	2.2±0.2* (+29%)	1.3±0.7* (31%)
185	33.0±0.51* (+47%)	2.27±0.11* (+34%)	1.8±0.37* (+39%)
370	37.9±1.5* (+69%)	2.9±0.9* (+70%)	2.3±0.2* (+77%)

Table 3. Rats were injected intraper-itoneally with indicated dose of aluminium daily for 60 days and treated as described in (table1).

Table 4 :Study of the effect of aluminium on serum bilirubin level in male rat over 30 and 60 days.

Aluminium dose µmoles/kg BW	Serum bilirubin(mg/dl) 30(days)	60(days)
None	0.45±0.1	0.44±0.07
35	0.60±0.03* (+33%)	0.60±0.20* (+36%)
185	0.68±0.06* (51%)	0.78±0.1* (+77%)
370	0.84±0.13* (+86%)	1.1±0.01* (+50%)

Table 4. Rats were injected intraperi-toneally with indicated dose of aluminium daily for 30 and 60 days respectively. Sera were collected as described in Materials and Methods Serum bilirubin concentration was expressed in mg/dl. Each data is the mean ISD of mean±SD five separte experiments. Statistically significant defference from the controls where $P<0.05$.(*)

Last experiment carried out was the effect of aluminium on the serum bilirubin. Rats were injected with varying amounts of aluminium and serum bilirubin level was determined and results are presented in (table 4). Administration of 35, 185, 370 μmoles/kg BW aluminium daily for 30 days elevated serum bilirubin level by 33,51% and 86% respectively in comparison to un-injected aluminium groups. Next 35,185 and 370 μmoles/kg of aluminium was injected daily for 60 days. The results obtained are presented in (table 4). Showing 36%, 77% and 150% elevations in serum bilirubin concentrations in comparision to controls.

DISCUSSION

Previously a number of publications from different laboratories throughout the world indicated that aluminium in blood circulation binds to serum transferrin[15,16] and interferes with a number of biochemical pathways including heme synthesis[17], lipid and carbohydrate metabolism [10,18]. Transferrin is taken up by hepatocytes and gives up is associated iron into the cells for synthesis of heme and non heme proteins[19]. Aluminium-transferrin as well as iron-transferrin complex is taken up by hepatocytes. Aluminium is accumulated in the nuclei and other subcellular organells which may cause damage to the cell copartments. The accumulation of aluminium was reported to be a time and dose dependent processes[9]. Data which have been presented in this manuscript showed that aluminium administration to rats lead to the elevations of serum LDH,AST and ALT levels (table 1,2,3). The elevations of these serum enzymes were dependent on the time and amount levels of aluminium administration. Our data are in good agreement with previous report[9]. Suggesting that aluminium may cause damage to cell membrane and lead to the leakage of the enzymes into the extracellular media. The elevation of total serum bilirubin in our study may suggest the disturbances of liver function and increased red cell destruction which may also lead to the production of bilirubin. The elevations of serum LDH,AST, ALT and bilirubin following aluminium administration might be consider as suitable indexes for liver dysfunction. Administration of aluminium may also stimulate liver for protein production. Significant elevation in serum protein level was seen in aluminium treated animals Data have not been shown but used for expressing enzyme specific activities in this study.

More investigations are needed to elucidate the exact mechanism by which aluminium might disturb hepatocytes function.

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