INTERFERENCE OF ALUMINIUM WITH CARBOHYDRATE METABOLISM IN MALE RATS: A MODEL STUDY OF DIALYSIS PATIENTS

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Abstract

The effects of aluminium and aluminium-citrate on some serum parameters related to carbohydrate metabolism in male Wistar rats were investigated. Daily intraperitoneal administrations of 1 mg/kg body weight of aluminium or aluminium-citrate (1: 1) for 50 days led to the elevation of serum insulin by 24 or 40 percent respectively, with the concomitant decrease in serum glucose concentrations by 17 and 14 percent. Serum insulin levels showed reduction by 36 and 29 percent following daily treatments of animals with 2 mg/kg aluminium or aluminium-citrate (1: 1). Serum glucose concentration elevated by 14 and 16 percent. Daily injections of 2mg/kg aluminium without citrate led to the reduction of liver glycogen by 60 percent, whereas if aluminium (2mg/kg) is injected in complex with citric acid this effect is reversed. The effect of aluminium toxicity and the importance of citrate in this aspect is discussed.

Introduction

Recent observations show that aluminium in dialysis fluid transfers across dialysis membrane, enters blood circulation and causes a number of pathophysiological disorders including bone disease [1], hypochromic microcytic anemia [2] and neurological disorders [3]. It has been reported that the metabolism of carbohydrate might be affected in aluminium intoxication through the inhibition of some kinase enzymes involved in metabolic pathways [4]. Hexokinase is a Mg-ATP dependent enzyme that catalyses the first step in glucose utilization by transferring the terminal phosphoryl moiety from its preferred substrate $\beta-\gamma$-Mg-ATP to form glucose 6-phosphate with the production of Mg-ADP [5]. Al-ATP is a competitive inhibitor of hexokinase against the natural substrate, Mg-ATP [6]. Womack and Colowick showed that the inhibition of hexokinase activity was related to aluminium contamination of the commercial ATP [4]. A report by Lia and Blass indicates that the activities of rat brain glycolytic enzymes including hexokinase, phosphofructo-kinase, pyruvate kinase and lactate dehydrogenase are affected by aluminium [7]. The participation of ATP-in carbohydrate matabolism and the interference of aluminium with ATP- dependent enzymes as well as the role of citrate as a stimulator of aluminium absorption [8] led us to investigate the effect of aluminium in the form of free and/or complexed with citrate on some serum carbohydrate related parameters in male rats.

Keywords: Aluminium, Citric acid, glucose, insulin, glycogean

Materials and Methods

Chemicals: All chemicals were reagent grade and purchased from Sigma Chemical Company. Throughout

this study, the glassware were soaked overnight in 1 M nitric acid and rinsed with distilled and double distilled water in order to minimize any metal contamination.

Preparation of aluminium-citrate complex: Aqueous stock solutions of aluminium (20 mg/ml) as aluminium chloride and/ or sodium citrate (20 mg/ml) were prepared separately. Equal volumes of each solution were mixed and the pH was made to 7.4 with NaOH. The concentration of aluminium in the final solution was 10 mg/ml. Aliquots of the prepared solution were administered to the animals to obtain indicated doses of aluminium.

Animals and treatment: Male Wistar rats weighing 200-250 gr, obtained from the Pasteur Institute Tehran, were used. Healthy animals were maintained on food and water at the standard conditions.

For this study, indicated doses of aluminium or aluminium in complex with citrate were injected intraperitoneally for 50 days. Animals treated with only saline were considered as the control group. At the time of the experiments (Day 51), animals (6 in each group) were anesthetized and blood samples were withdrawn from their hearts and allowed to clot. The sera were then separated by centrifugation and used for the analysis. Plasma biochemistry: Serum glucose was determined by orthotoluidin method [9]. Liver glycogen was measured using the method of Kemp and Kits [10] and the activity of amylase was assayed by the method of Caraway [11]. Serum insulin was measured by immunoradiometric assay using laboratory kits purchased from Diagnostic Product Corporation (Los Angles CA 90045). LKB gamma counter (model 1275) was used to measure the radioactivity.

Results

The effect of aluminium and aluminium-citrate on the concentrations of serum glucose, insulin, amylase and liver glycogen was studied, the results of which are shown in Table 1. Daily administration of 1 mg/kg aluminium as $AlCl_3$ for 50 days led to an increase in serum insulin level by 24 percent and in liver glycogen by 30 percent. These elevations were accompained by 17 percent decrease in serum glucose level. The activity of amylase remained unchanged.

When aluminium (1mg/kg) was administered as a complex with citrate (1: 1), it was found that serum insulin levels elevated by 40 percent whereas glucose levels decreased by 14 percent. No significant changes were observed in liver glycogen content and serum amylase activity.

Administration of 2mg/kg aluminium, in contrast to the above results, decreased serum insulin level and liver glycogen content by 36 and 60 percent respectively. Serum glucose level and amylase activity were elevated by 14 and 15 percent respectively. When aluminium (2mg/kg) was administered in association with citrate

Table 1: Changes in the concentrations of serum glucose, insulin, amylase and liver glycogen following administration of aluminium or aluminium citrate complex in rat. For details see text.

Values are mean ± SD of six separate experiments performed in duplicate. In parentheses, the percent increase and/ or decrease from the mean values for control is shown.

Treatment	glucose (mg/100)	insulin (uIu/ml)	amylase (Iu/lit)	Liver glycogen (mg/g)
Control	99.5±14.2	18.4±4.2	1488.5±38.9	22.5±1.2
Al (1mg/kg)	82.4±18.4 (-17%)	22.9±3.2 (+24%)	1477.1±56.0 (-1%)	29.54±0.9 (+31%)
Al (lmg/kg) +Citrate	86.0±3.9 (-14%)	25.9±2.3 (+40%)	1465.5±54.5 (-2%)	23.3±2.6 (+3%)
Al (2mg/kg)	113.2±13.4 (+14%)	11.8±2.6 (-36%)	1714.6±19.4 (+15%)	9.2±2.8 (-60%)
Al (2mg/kg) +Citrate	114.6±17.3 (+16%)	14.2±3.5 (-22%)	1698.8±25.3 (+14%)	24.2±1.6 (+7%)

(1:1), although these changes in glucose and insulin levels still persist, the amount of liver glycogen was restored and showed no significant difference with the controls.

Discussion

ATP as well as ADP exist under physiological conditions primarily as the Mg^{2+}-Chelate, and it is this form of the nucleotide which acts as a substrate for most of the ATP utilizing enzymes including hexokinase, phospho-fructokinase, etc. [7]. Aluminium shows high affinity to interact with ATP molecule [12]. Furumo and Viola in 1989 reported that aluminium interferes with creatin kinase reaction by interacting with ATP molecule [13]. The importance of ATP in carbohydrate metabolism on one hand, and its affinity to bind aluminium on the other, may explain the mechanism by which carbohydrate metabolism is disturbed by aluminium. The inhibition of hexokinase, a key enzyme in glucose metabolism, by aluminium as reported by Solheim and Fromm [14] leads to the decreased glucose uptake and consumption by the cells which is in good agreement with the present observations. Administration of 2 mg/kg aluminium increased serum glucose level by 14 percent which was accompanied by 36 and 60 percent reduction in serum insulin and liver glycogen. Alternatively aluminium might have interferred with beta cells' function with the consequent decrease in insulin secretion and glucose elevation. Similar experiments have revealed the inhibitory effects of aluminium on hormone secretion. It has been reported that aluminium diminished the secretion of parathormone from the parathyroid gland [15].

Administration of aluminium (1mg/kg) showed an opposite effect on glucose, insulin and glycogen levels when compared with the dose of 2mg/kg. This result indicates that aluminium, like some other metal ions, could act by two different mechanisms. The dual effects of aluminium as activator and inhibitor of adenylate cyclase in the liver has been previously reported [16].

Citric acid has been reported to be a useful chelating agent, enhancing aluminium absorption by the intestinal mucosal cells and increasing serum aluminium concentration [8]. In our study it was interestingly observed that concomitant administration of citrate with aluminium could reverse the reducing effect of aluminium on the liver glycogen level. Aluminium in blood circulation is present in either transferrin bound or free forms [17]. It has been reported that citric acid facilitated the binding of aluminium to serum transferrin [18]. Thus, in the presence of citrate, more aluminium could be found as transferrin bound. Aluminium has been reported to be taken up by the cells either as transferrin bound and/or free aluminium [19]. Free aluminium is a toxic agent and when entering the cell may disturb the cell functions.

Since the liver glycogen content remains unchanged when citrate is present, it could be concluded that in the presence of citrate less aluminium is present in free form and thus less is absorbed by the cells. Alternatively, citric acid has been known to be a proper substrate for the krebs cycle and its administration to rats may lower the degradation of reserved carbohydrate which is otherwise influenced by the free aluminium.

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