ALUMINIUM UPTAKE BY RAT ISOLATED HEPATOCYTES AND ITS EFFECT ON MITOCHONDRIA OXYGEN UPTAKE

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SUMMARY: The aluminium uptake by rat isolated hepatocytes and its effect on oxygen uptake has been investigated. The hepatocytes aluminium content has elevated after incubation in medium containing aluminium-transferrin (Al-tf) or free aluminium (ultrafiltrable). The free aluminium (Al) influx to the cell is two times more than aluminium bound transferrin. The aluminium uptake from transferrin depends on the degree of saturation of transferrin depends on the degree of saturation of transferrin with AI. Aluminium transferrin complex and ultrafiltrable fractions of aluminium lead to the reduction of oxygen uptake by 18 and 32 percent respectively. Cellular uptake of aluminium and its effect on mitochondria function has been discussed.

Key Words: Aluminium, Al-transferrin, hepatocytes, oxygen uptake.

INTRODUCTION

Aluminium accumulation in patients undergoing renal dialysis is associated with development of a number of pathophysiological disorders including hypochromic microcytic anemia (1) dialysis osteomalacia (2) and neurological disorders such as dialysis dementia (3) and Alzheimer's disease (4).

The major sources of aluminium contamination in these patients come from dialysis concentrate, the water uses for dilution of this concentrate, storage containers and aluminium phosphate binder agents use by these patients (5). Aluminium from these sources enters blood stream and causes toxicity. The major forms of aluminium in blood circulation has been reported to be in complex with transferrin using immuno-affinity chromatography (6) and spectrophotometric titration techniques (7). Transferrin is a single chain glycoprotein and responsible for the transportation of iron across blood circulation (8).

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The possible mechanism by which aluminium causes anemia in dialysis patients may be due to the inerferences of this metal with iron binding to transferrin (9).

The probable mechanism by which aluminium causes neurological disorders is still a matter of dispute. It has been reported that aluminium content of chromatin part of nuclei in neurons of Alzheimer's patients with aluminium overload is much higher than Alzheimer's patients without aluminium (10). It seems that aluminium is taken up by the cells and distributes in subcellular fractions and influences biochemical pathways. To our knowledge the uptake of both forms of aluminium (protein and non-protein bound fractions) by the cells has not been reported. Therefore, the aim of the present study was to undertake the aluminium (free and protein bound fractions) uptake by hepatocytes and its influences on mitochondria oxygen uptake.

MATERIALS AND METHODS

All chemicals used in this project were purchased from BDH chemicals ltd (U.K) unless otherwise staded. They were the best quality.

In order to avoid metal contamination throughout this study, all glassware were pre-washed with 1M nitric acid and washed with 1M nitric acid and washed with distilled and deionized water. Plasticware were pre-washed with 10 mM EDTA and washed with distilled water and deionized water. The aluminium content of deionized water was less than 1 ug/L as determined by flameless atomic absorption.

Preparation of aluminium-transferrin complex

Apo-transferrin was prepared from human transferrin (HOECHST) by the method of Morgan (11) and saturated with aluminium by addition of 0.09 mmole/L of aluminium potassium sulfate in 1.8 mmole/L citric acid to transferrin (2 mg) dissolved in Earle's medium. Excess aluminium was removed by dialysis. The final aluminium content of aluminium-tranferrin complex was 2 ug/mg protein. Protein and aluminium concentrations were determined by methods of Lowry (12) and Parkinson *et al.* (13).

Preparation of rat liver mitochondria

Male Wistar rats were sacrificed by decapitation. Their livers were removed, trimmed, and rinsed with cold aluminium free 0.25 M sucrose. They were blotted dry, weighed, chilled and homogenized with 5 volumes of ice cold 0.25M sucrose. This and other subsequent steps were carried out at 4°C. The homogenated was first filtered through nylon mesh and then centrifuged at 2000 g to remove nuclei and intact cells. The supernant was centrifiged at 11700 g for 20 min in M. SE-18 centrifuge. The supernatant was discarded and the pellets were resuspended in 10 volumes of 0.25 M sucrose and recentrifuged at 11700 g. The succinic dehydrogenase activity was determined by the method of (14). Rat isolated hepatocytes were kindly gift from Dr. L. Agius from department of Medicine of Newcastle University.

Oxygen uptake by rat liver mitochondria

Rat liver mitochondria were prepared as described above and suspended in 2 mM HEPES buffer pH 7.2 containing 0.25 M sucrose at a concentration of 40-50 mg/ml of protein. The oxygen uptake by mitochondria was investigated by using yellow Spring Oxygen electrode. 3.8 ml of incubation medium (120 mM KCl, 10mM HEPES, 5 mM $MgCl_2$, 5 mM Na_2PO_4, pH 7.2) and 40 ul of substrate (IM malate, 100 mM glutamate) was added to the cell and stirred at 30°C. The position of oxygen electrode was carefully adjusted so that its tip made contact with the medium, care being taken to exclude any air bubble. 100 ul of suspension of mitochondria was then added to the cell and the oxygen uptake by the mitochondria was recorded. 10 ul of ADP (100 mM) was added and when recorder trace levelled-off (20 secs), second pulse of ADP was added. When the recorder trace again levelled-off (20 secs) 70 ul 2,4 dinitrophenol (10 mM) was added and the oxygen monitored.

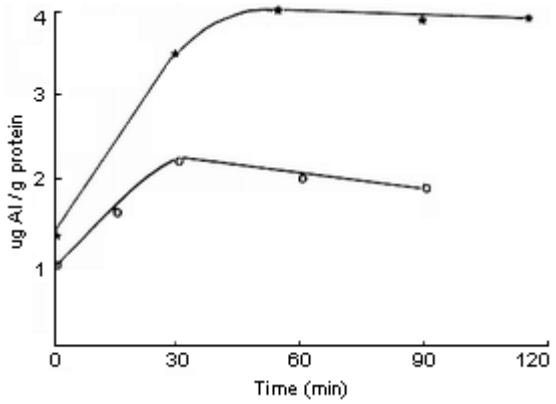

Figure 1: Time progress for aluminium uptake by rat hepatocytes at pH 7.4. * Hepatocytes incubated in medium containing 160 ug/L. Al as Alk (SO_4). O Hepatocytes incubated in medium containing 160 ug/L Al as Al-tf. Each point is the mean of three observations.

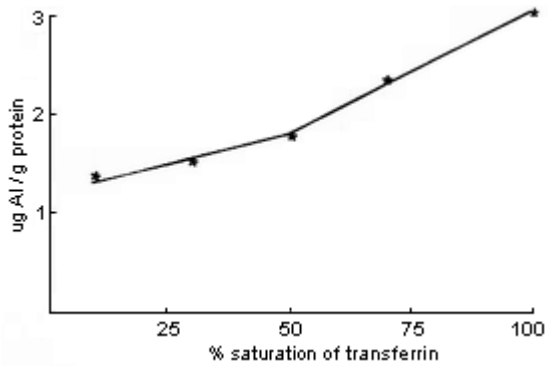

Figure 2: Effect of increasing saturation of transferrin with aluminium on the uptake of Al by rat hepatocytes at pH 7.4. Each point is the mean of six observations.

RESULTS

Uptake of aluminium by isolated hepatocytes

Isolated hepatocytes were incubated at 37°C in Earle's medium pH 7.4 containing 160 ug/L aluminium as aluminium potassium sulfate. At appropriate time intervals aliquots of the hepatocytes suspension were with-drawn and put in tubes immersed in an ice bath. The cold sus-

Table 1: Effect of aluminium on the ratio of ADP/O of rat liver mitochondria.

	Control	Al-tf*	Al*
	2.85 ± 0.19	2.35 ± 0.12	1.96 ± 0.15
ADP/O	(4)	(6)	(5)

* Aluminium was added prior to the addition of lst ADP. Number of experiments are shown in parentheses.

pension was centrifuged at 2500 g for 5 min and supernatant discarded. The hepatocytes were resuspended in cold 0.9 g/100 saline and washed three times with centrifugation. The pellets were dissolved in 10% W/V Triton-X-100 in which the aluminium and protein content were determined. The results obtainedare shown in Figure 1. Where the aluminium content of the hepatocytes increases from 1.3 ug/L protein on mixing to 4 ug/g protein after 1 h, remaining at that level during a further 1 h incubation. When aluminium presented to the hepatocytes in the from of fully saturated as a complex with aluminium, there was an increased in aluminium content of hepatocytes reaching a maximum of 2.25 ug/g protein after 30 min incubation but decreasing to 1.9 ugAl/g protein after a further 60 min incubation Figure 1. when hepatocytes were incubated for 2 h with transferrin containing different proportion of fully saturated aluminium transferrin and apotransferrin the aluminium content of the hepatocytes increased as the aluminium content of the transferrin rose Figure 2.

Effect of aluminium on oxygen uptake by mitochondria

The effect of aluminium on oxygen uptake by mitochondria was studied. Rat liver mitochondria was prepared and characterized as mentioned in Materials and Methods. The effects of both free and Aluminium bound transferrin on oxygen uptake by mitochondria were studied. When 160 ug/L of aluminium as aluminium potassium sulfate was added to the mitochondria-containing medium the ADP/O ratio fell from 2.85 to 1.96 Table 1.

When fully saturated aluminium transferrin was added to the medium the ADP/O ratio fell from 2.85 to 2.35. It is likely that free aluminium had more effect on oxygen uptake by mitochondria in comparison to protein bound.

DISCUSSION

Although aluminium is now widely recognized as a toxic agent in chronic renal failure patients maintained on regular hemodialysis the exact mechanism of its toxicity remains to be elucidated. Our previous studies showed that aluminium bound to serum transferrin (15) and the complex of aluminium-transferrin interacts with the same receptors as iron-transferrin (16). The present study demonstrated that when hepatocytes were incubated for 2 h in Earle's medium pH 7.4 containing 160 ug/L of aluminium, the aluminium uptake increased for up to 45 min and the levelled-out. Hepatocytes also incubated with transferrin fully saturated with aluminium-transferrin for 90 min and also transferrin in complex with aluminium with differing degree of saturation (10-100%). The uptake of aluminium from fully saturated Al-tf reach a maximum after 30 min but was less than when hepatocytes were incubate with free aluminium. This study shows that aluminium uptake from free aluminium or a Al-tf was dependent on time of incubation. These data confirms observations of Muller and Wilhem that hepatocytes take up aluminium (0-20 uM) (17) and this process stimulated when 50 uM of Cadmium was added to the medium (18). It should be noted that they have used much higher aluminium concentrations than we used. In addition to that they have only studied the uptake of free aluminium and its uptake by hepatocytes (17). Previously we have found that aluminium in the cell is distributed in subcellular fractions mostly in nuclei and mitochondria (9). It was therefore necessary to see whether aluminium had any effect on mitochondria function. Heme synthesis was reduced in the presence of aluminium (19). In the present study when the effect of free and transferrin bound aluminium on oxygen uptake by rat liver isolated mitochondria was investigated, there was 18 and 32 percent reduction of oxygen uptake by free and transferrin bound aluminium respectively. These observations are in good agreement with those of Heffron *et al.* (20) who have found that oxygen reduction was observed when mitochondria were incubated with aluminium. We have used malate and glutamate substrate *in stead of* succinate which has been by them. However, both investigations lead to the same conclusions, but still needs more study to elucidate the exact mechanism of aluminium toxicity.

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