ALUMINUM DISTRIBUTION IN RAT LIVER SUB-CELLULAR FRACTIONS IN RELATION TO NEUROLOGICAL DISEASE IN HEMODIALYZED PATIENTS

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SUMMARY: The distribution of aluminum within the sub-cellular fractions of rat liver was investigated. The majority of the aluminum was bound to nuclei and mitochondria. The aluminum content of nuclei and mitochondria was 5.2 and 4.9 µg per gram protein respectively. Lysosomes, cytosol and microsomes contained 3.75, 1.12 and 0.86 µg aluminum per gram protein, respectively. In the nuclei the majority of aluminum was in the non-DNA compartment of nucleoprotein and SDS-PAGE showed that histones could bind aluminum. The relationship between aluminum toxicity and neurological disorders in chronic renal failure maintained on hemodialysis has been discussed.

Key Words: Aluminum, hemodialyzed patients, sub-cellular localization.

INTRODUCTION

Aluminum is an ubiquitous element, comprising approximately 8 % of the earth's crust (1). Normal adults consume approximately 3-5 mg aluminum in the daily diet and variable amounts from drinking water depending on local conditions including alum treatment and acidification (2). The gastrointestinal absorption of aluminum from aluminum phosphate binders has also been reported in those who use aluminum as antacids (3). In chronic renal failure patients with end-stage renal disease who are maintained on regular hemodialysis, aluminum is transported from dialysis fluid, enters blood circulation and binds to serum transferrin (4). Transferrin is a ß-glyco-protein and is responsible for the transportation of iron from it's site of absorption to the site of utilization in the cells (5).

The binding of aluminum to serum transferrin was first reported by G. A. Trapp in 1983 (6). His findings were then confirmed by a number of other laboratories (7,8). Similar to iron-transferrin complex, aluminum-transferrin also binds to the same receptors at the placental plasma membrane, internalized to the cells (9).

Aluminum has been suggested to be a causative factor in dialysis encephalopathy (10) dialysis osteodystrophy (11) and a microcytic hypochromic anemia (12). It has also been implicated in the etiology of Alzheimer's disease (13). After repeated injections of aluminum lactate over 60% of the aluminum body burden was in the liver, about 25% in bone, about 10% in the spleen and the remainder in numerous other organs of rabbits. Similar aluminum distribution has been reported for human (14).

In patients with dialysis dementia, aluminum is located in the cytoplasm of the neurons whereas in Alzheimer's disease aluminum is found to be associated with the nucleoli (10).

The present project was undertaken to investigate the normal distribution of aluminum in rat liver sub-cellular fractions.

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MATERIALS AND METHODS

Experiments were done with male Wistar rats. The rats weighing between 200-250 grams were starved for 15 hours prior to experiments. They were killed by cervical dislocation. The livers were immediately removed, trimmed and rinsed, blotted dried, weighed, chilled and placed into 5 volumes of ice-cold 0.25M sucrose. This and all other subsequent steps were carried out at 4°C. The tissue homogenate was filtered and nuclei, mitochondria, lysosomes, microsomes and the cytosol were prepared according to the method of Fleischer and Kervina (15).

The prepared nuclei, mitochondria, lysosomes and microsomes were used for protein (16) and aluminum determinations (17). Rat liver chromatin and DNA were prepared by the method of Schrader *et al* (18). DNA concentration was determined flourmetrically by the method of Pecq and Poaletti (19). Enzyme markers including succinate dehydrogenase was measured by the methods of Cooney and Dowson (20). Alkaline phosphatase and lactate dehydrogenase activities were determined colorimetrically by the routine laboratory methods (21,22). Deionized water was used for the preparation of the fractions and also for other purposes, including washings and preparation of solutions. All chemicals were reagent grade and purchased from Sigma Chemical Company.

Sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was carried out as reported elsewhere with a minor modification of stacking gel (10-12%) and running gel (16-18%) with discontinuous buffer system (8). Calf thymus histone was received as gift from Dr. L Aguies in U.K.

RESULTS

Aluminum content of sub-cellular fractions

Distribution of aluminum within the sub-cellular fractions of rat liver prepared as described in the methods was examined. The fractions were prepared in 0.25M sucrose and the enzyme marker activities were determined. The data are shown in (Table 1). Protein patterns obtained after SDS-PAGE of each fraction was also identified and were clearly differential (Figure 1), suggesting that each individual fraction was provided with no protein contamination and could be used for further experiments.

Aluminum determinations of each individual fraction was carried out using Triton X-100 to solublize the fractions. The aluminum content of the homogenate ranged from 1.56-4.04 μg per gram protein. Nuclei prepared from the homogenate contained 5.2 μg aluminum per gram protein which is 27% of the aluminum in the homogenate. Mitochondria were prepared from the supernatant obtained after centrifugation of the original

Table 1: Measurement of marker enzymes in rat liver sub-cellular fractions.

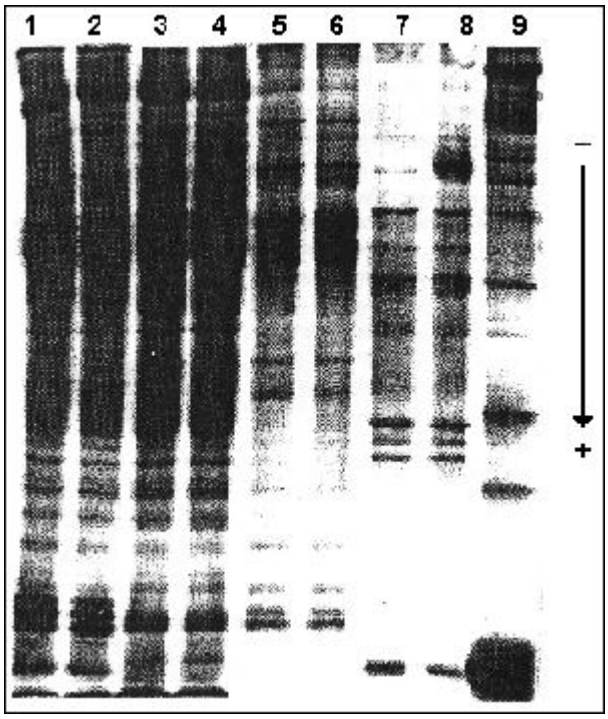
Fraction	5'-Nucleotidase Enzyme unit/g protein	LDH Enzyme unit/g protein	SDH O.D/g protein/30 min
Homogenate	19.60 (4)	2436.50 (5)	0.23 (2)
Nuclei	25.26 (3)	592.09 (5)	0.08 (2)
Mitochondria	21.15 (4)	552.25 (5)	0.29 (2)
Lysosomes	28.00 (2)	556.61 (3)	-
Microsomes	48.36 (3)	1163.68 (4)	0.03 (2)
Cytosol	4.77 (4)	3716.83 (3)	0.05 (2)

The number of rats are given parantheses.

Table 2: Distribution of aluminum among the sub-cellular fractions of liver from male rats.

Fraction	mg Al/gram liver	% Aluminum
Homogenate	2.80 (7)	
Range Nuclear	(1.56-4.04) 5.20 (7)	27.00
Range Mitochondria	(2.40-8.90) 4.90 (4)	27.00
Range Light mitochnodria (Lysosomes)	(3.40-5.80) 3.75 (2)	
Range Microsomes	(1.40-1.60) 1.12 (5)	4.70
Range Cytosol	(0.75-1.40) 0.86 (5)	7.60
Range	(0.38-1.37)	

Figure 1: SDS-PAGE of rat liver sub-cellular fractions
1,2: Nuclei; 5, 6: Microsomes; 9: Human erythrocyte
3, 4: Mitochondria; 7, 8: Cytosol; membrane (marker) protein.



homogenate at 2000 g and they contained 4.9 μg aluminum per gram protein. Lysosomes contained 3.75 μg aluminum per gram protein. Microsomes and cytosol each contained 1.12 and 0.86 μg aluminum per gram protein respectively (Table 2). It is obvious that the aluminum content of the nuclear and mitochondrial fractions should be considered further due to the high concentration of aluminum in these fractions.

Table 3: Measurement of aluminum content of whole chromatin and DNA from four separate rat liver nuclei. Aluminum ng/rat liver.

Chromatin	DNA	% Aluminum in DNA
786	288	37
660	196	30
1019	266	26
648	154	24
Mean±SD 778±172	226 ± 62	29±5,7

Each value was the man of three reading of absorption by atomic absorption.

Aluminum in the nucleus

As the nuclear fraction contained significant amounts of aluminum the distribution of aluminum within the nucleus itself was then determined. Nuclear fractions were prepared as described earlier. The DNA and protein content of the chromatin was determined. As an additional control of the protein patterns, they were determined by SDS-PAGE and found out to be identical to that in the literature, pattern labeled according to the nomenclature proposed by Davie (23). The DNA was then extracted from the nuclear proteins using chloroform-isoamyl alcohol and collected by cen-

Table 4: Measurement of aluminum content of whole chromatin and DNA from male rat liver nuclei. Aluminum μg/g DNA.

Chromatin	DNA
4003	330
(4)	(4)

The number of rat livers are shown in parntheses

trifugation. The aluminum content of whole chromatin was determined and the results are shown in Tables 3 and 4. Most of the aluminum could not be extracted with the DNA and might be bound to nucleoprotein.

Comparative binding of aluminum and iron to histones

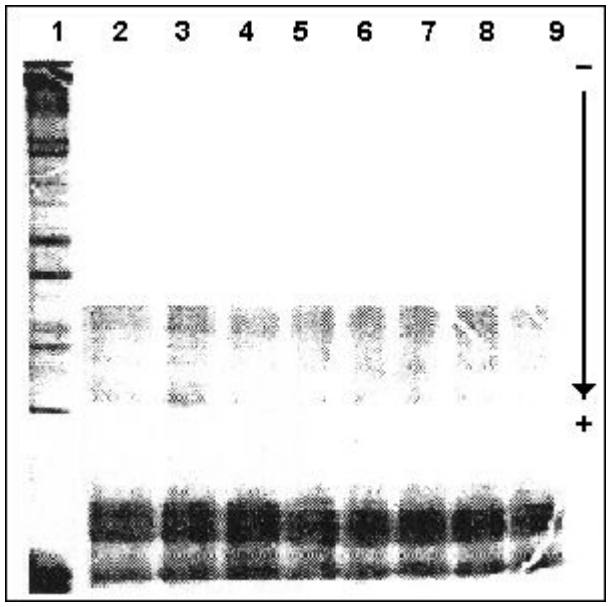
As histones form the major protein compartment of the chromatin, it becomes necessary to investigate the possible interaction between aluminum and these proteins. For this purpose, calf thymus histone was dissolved in 5 ml of Earle's buffer (pH 7.4) to give a solution containing 2.5 mg/5 ml. Complex of iron and/or aluminum with citric acid was prepared separately as mentioned elsewhere (8) and were added to histone solutions. The final concentration of aluminum or iron was 2000 μg per gram protein. The solutions were mixed thoroughly and left at room temperature for 4-6 h. The protein metal solutions were then dialyzed against 100 volumes of 0.02 M $NaHCO_3$ to remove unbound metals and the Aluminum or iron content were then determined. The iron content was 1200 μg/g protein and the aluminum content was 1440 μg/g protein.

The protein solutions were then subjected to SDS-PAGE.

Comparison of the patterns given by the untreated histone, the citric acid treated protein and that to which the iron and/or aluminum citrate complex had been added at a concentration of 2000 μg/g protein, showed that the protein to which iron and aluminum had been added, there was a reduction in one of the components (H3) (Figure 2).

In another experiment aluminum and/ or iron citrate solution added to histone at a concentration of 3000 μg/g protein and the solutions were then dialyzed to remove unbound metals and was then examined by SDS-PAGE, it was found that the histone to which iron had been added contains 1850 μg/g of protein and that to which aluminum had been added contains 2380 μg/g protein. The untreated histone gave a pattern similar to the faster moving protein of rat liver chromatin. The pattern given by the iron containing protein showed only a reduction in the intensity of protein H3 as for the histone containing only 1150 μg/g protein. However, for the solution containing 2380 μg/g aluminum protein

Figure 2: SDS-PAGE of calf thymus histones
1:Marker protein;
2, 3:Histones;
4, 5:Histone treated with citric acid;
6, 7:Iron-histone; 8, 9: Aluminum-histone.

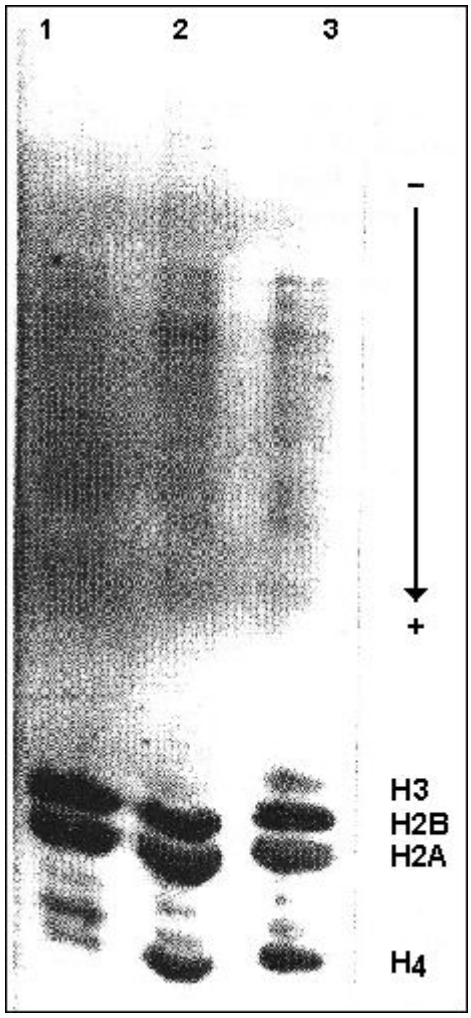


was there a reduction not only in H3 but also in H4 with an increase in a band with slightly lower mobility (Figure 3).

DISCUSSION

Histochemical studies indicated that aluminum in the brain of rabbits and cats was located dominantly in the nuclei, especially bound to the chromatin (24). Liver aluminum is selectively taken up by nuclei where it combines with DNA (25). On the other hand, investigations of the sub-cellular distribution of aluminum in postmortem examination of the brain have shown that in neurons of dialysis dementia patients the aluminum was located largely in the cytoplasm, whereas in the neuron of dialysis Alzheimer's disease it was located largely in the nucleus (25). None of these results were based on quantitative chemical analysis but relied on histochemical staining techniques and therefore it was decided to examine the distribution of aluminum among the sub-cellular fractions of male rats using flameless atomic absorption spectrophotometry. The results obtained showed that the majority of the aluminum was accumulated in the nuclear and mitochondrial fractions, with the distribution of aluminum among the cytosol,

Figure 3: SDS-PAGE of calf thymus histone
1: Aluminium-histone;
2: Iron-histone;
3: Histone.



microsomes and lysosomes all being approximately one forth of that in the nuclear fraction. These findings therefore agree with the earlier histochemical observations of Kushelvsky *et. al.* 1976 (26) and De-Bonie *et. al.* 1974 (24).

This apparently high affinity of nuclei and mitochondria for aluminum prompted another study where nuclei from rat livers were isolated chromatin and DNA were extracted from separate livers (18). The concentration of aluminum in the chromatin was found to be 4030 μg/g DNA but only 330 μg/g DNA in the chloroform-isoamyl alcohol extract. Thus more than 90% of the nuclear aluminum was not bound to DNA. These observations are consistent with the findings of Sigel (27) who after inducing encephalopathy in rats by injecting aluminum showed that 80% of the aluminum in liver was recovered from the chromatin. Control nuclei contained 1550 μg aluminum/g DNA and aluminum-treated animal 5700 μg aluminum/g DNA.

Karlike *et. al.* (1980) has earlier described interaction between aluminum and DNA and suggested aluminum might form at least three Complexes with DNA with the ratio of Al/DNA within the range 0.001-0.7. The multiple species of aluminum which exist in aqueous solution could well lead to a variety of reactions with DNA. Exactly how aluminum binds to DNA is not yet certain, but phosphate group of DNA may well be involved (28).

Crapper-Maclachlan provided evidence of an interaction between aluminum and DNA (29).

Not all the aluminum in chromatin is bound to DNA. A significant proportion would appear to be associated with histones, non-histones proteins and RNA. Wardle has suggested that Ca can displace histone from chromatin and interact with negatively charged phosphate groups of the DNA. There is evidence that aluminum displaces Ca from binding to acidic phospholipids in order of Al>Mg>Ca>Na (30). The electrophoretic patterns of histones incubated with iron or aluminum at a concentration of 2000 μg/g histone protein showed that addition of these metals to the protein resulted in deletion of the H4 histone from the electrophoretic patterns. When the concentrations of the metal ions was increased to 3000 μg/g protein the electrophoretic pattern of iron-histone remained unchanged whereas with the aluminum histone the position of histone H3 in addition to H4 was also changed. These changes might be due to either the cross-linking or hydrolyzing of the proteins caused by aluminum or iron. In relation to neurological disorders which appear in chronic renal failure patients with aluminum intoxication the exact mechanism by which this toxic element causes encephalopathy and Alzheimer's disease is still unclear. Aluminum may interfere with transcription or translation of some special proteins in the neurons of aluminum intoxicated patients. However more investigation should be carried out to elucidate this speculation.

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