PROTEIN BINDING OF ALUMINIUM IN NORMAL SUBJECTS
AND IN PATIENTS WITH CHRONIC RENAL FAILURE

H Rahman, S M Channon, A W Skillen, M K Ward, D N S Kerr
Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Summary

Ultrafiltration of serum through YM10 membranes showed that 46 per cent of the aluminium in normal subjects and 33 per cent of the aluminium in patients with chronic renal failure is ultrafiltrable, suggesting that the majority of the aluminium is bound to some serum component(s) having molecular weight greater than 10,000 daltons. After desferrioxamine infusion, both the ultrafiltrable and protein-bound aluminium increases significantly, probably due to mobilisation of aluminium from body tissues. Gel filtration on Sephacryl S-300 and affinity chromatography have shown that transferrin is the major aluminium binding protein.

Introduction

Aluminium accumulation in chronic renal failure has been implicated in the causation of dialysis dementia, dialysis osteodystrophy and microcytic hypochromic anaemia but very little is known about the mechanism(s) by which aluminium causes its toxic effects.

During haemodialysis aluminium is transferred from the dialysis fluid to the patient against a concentration gradient and is difficult to remove subsequently [1]. This is probably due to the tight binding of aluminium to some serum component(s). Although the possibility of removal of aluminium by dialysis with low aluminium-containing dialysis fluid is controversial, a significant amount of aluminium can be removed by haemodialysis after desferrioxamine infusion [2], although the mechanism by which this occurs is not well defined.

Studies are carried out to determine the protein binding of aluminium in normal subjects and in patients with chronic renal failure and to determine the effect of desferrioxamine on the protein binding of aluminium.
Materials and methods

Protein binding of aluminium in serum was studied by ultrafiltration, gel filtration and affinity chromatography.

Ultrafiltration was carried out in a stirred Amicon ultrafiltration cell which was pressurised by 5% CO₂ in air, using YM10 membranes (molecular weight cut off 10,000 daltons). Thirty normal subjects and 30 patients with chronic renal failure on haemodialysis were studied to determine the ultrafiltrable aluminium in the serum. Ten patients with chronic renal failure were studied before and 48 hours after an infusion of 2gm of desferrioxamine to determine the effect of desferrioxamine on the protein binding.

Specific aluminium binding protein(s) were identified by gel filtration and affinity chromatography. Gel filtration was performed in a Pharmacia column, packed with Sephacryl S-300 superfine. Earle’s Medium at pH 7.4 was the eluting buffer and the column was calibrated with IgM, IgG, transferrin, albumin and aluminium-desferrioxamine complex markers. The serum of five normal subjects and 15 patients with chronic renal failure on haemodialysis was studied by gel filtration to identify the aluminium binding protein(s) and another five patients with chronic renal failure were studied after a desferrioxamine infusion. Affinity chromatography was carried out with cyanogen bromide activated Sepharose coupled with anti-transferrin. Phosphate buffered saline at pH 7.2 was used in the immobilisation and washing cycle and phosphate citrate buffer at pH 2.8 was used in the dissociation cycle.

Aluminium estimations of the serum, ultrafiltrate and column fractions were performed by flameless atomic absorption spectrophotometry. For the fractions, matrix matched standards were essential and the background correction was needed for the fractions from the affinity column.

Protein estimations of the fractions were carried out by recording the absorbance at 280nm using an ultraviolet spectrophotometer, immunoturbidimetric assay on the centrifugal analyser (Cobas-Bio) and electrophoresis.

Precautions were taken to avoid aluminium contamination during the studies and analysis.

As some of the data were not normally distributed, non-parametric statistics were used for the analysis.

Results

In the normal subjects, the mean serum aluminium was 8μg/L (range 6.4–11.6μg/L). The mean ultrafiltrable aluminium was 3.6μg/L (range 2.8–4.5μg/L). The mean non-ultrafiltrable aluminium was 4.4μg/L (range 2.8–7.1μg/L). Forty-six per cent of the serum aluminium was ultrafiltrable. The remaining 54 per cent could not pass through the membrane and was probably bound to some serum component(s) (Figure 1).

In the patients with chronic renal failure, the serum aluminium (mean ± SD) was 99±54μg/L. The ultrafiltrable aluminium was 30±16μg/L and the protein-bound aluminium was 68±41μg/L. Thirty-three per cent of the serum aluminium was ultrafiltrable (Figure 1). The percentage of ultrafiltrable aluminium showed
ULTRAFILTRATION OF SERUM

![Graphs showing aluminium concentrations in normal subjects, CRF patients, before and after desferrioxamine infusion, and patients with CRF with and without aluminium binding proteins.](image)

Figure 1. Total serum, ultrafiltrable and ‘protein-bound’ aluminium in normal subjects, chronic renal failure patients and the effect of desferrioxamine on the protein binding of aluminium in patients with chronic renal failure.

A significant inverse correlation was observed between the total serum concentration of aluminium and the protein binding of aluminium in both normal subjects and patients with chronic renal failure (Spearman’s rank correlation coefficient: rs = -0.47, p < 0.01 for normals; rs = -0.52, p < 0.01 for chronic renal failure patients).

After desferrioxamine infusion, the serum aluminium (mean ± SD) showed a remarkable increase from 72±31μg/L to 198±94μg/L. Both the ultrafiltrable (21±7–81±33μg/L) and protein bound aluminium (51±25–117±63μg/L) showed a significant rise (Figure 1). The mean percentage ultrafiltrable aluminium showed a significant increase from 32 per cent to 43 per cent after desferrioxamine infusion (paired 't' test: t=6.81, p<0.001).

Gel filtration of serum from normal subjects and patients with chronic renal failure showed that aluminium was eluted as a single peak which was coincidental with the transferrin peak (Figure 2).

When gel filtration was performed with 2.5mg/ml and 5mg/ml of commercially available human transferrin (Behringwerke) before and after spiking with 200μg/L aluminium, the aluminium was eluted from the column as a prominent peak coincident with transferrin. However, when similar gel filtration studies were carried out with 5mg/ml and 50mg/ml of human albumin (Hoechst), no aluminium peak was detected in the column fractions coincident with albumin peak.
Gel filtration studies after desferrioxamine infusion showed that aluminium was eluted as two distinct peaks, the first coincident with transferrin peak and the second coincident with the aluminium-desferrioxamine marker peak on the column. During gel filtration, it was observed that the gel matrix absorbs aluminium from the buffer and serum ultrafiltrate. This aluminium can subsequently be taken up by the serum or desferrioxamine passing through the column. For this reason gel filtration could not be used for quantitative studies.

Affinity chromatography in five patients with chronic renal failure showed that about 35 per cent of the serum aluminium was eluted in the fractions from the washing cycle and the remaining 65 per cent was eluted in the dissociation cycle with the transferrin.

**Discussion**

In normal subjects 46 per cent (range 30–58%) of the serum aluminium is ultrafiltrable. This finding agrees with that of Lundin et al [3], but is in contrast with the finding of Elliott et al [4], who could not detect significant amounts of ultrafiltrable aluminium in normal subjects. In patients with chronic renal failure, 33 per cent (range 20–47%) of the serum aluminium is ultrafiltrable which is consistent with the finding of Elliott et al [4], but is higher than the 20 per cent ultrafiltrable aluminium reported by Graf et al [5]. The difference in the membranes used and conditions for the ultrafiltration studies between the workers [4,5] may explain this discrepancy. Our observation of an inverse correlation between the percentage ultrafiltrable aluminium and total serum aluminium agrees with the finding of Hosakawa et al [6].

After desferrioxamine infusion, the serum, ultrafiltrable and protein-bound aluminium showed a remarkable increase. The percentage of ultrafiltrable aluminium increased significantly. This finding is in agreement with that reported
by Graf et al [5] and suggests that desferrioxamine mobilises aluminium from the body tissues. The observation that the protein-bound aluminium also increased significantly was unexpected and suggests that some of the mobilised aluminium also binds with protein. The alternative possibility that aluminium-desferrioxamine complex is partly protein-bound is not supported by our column chromatography.

Gel filtration and affinity chromatography have shown that transferrin is the major aluminium binding protein. This finding is consistent with that of Trapp [7] and Cochran et al [8], but is in contrast with that of King et al [9], who found five aluminium peaks in their patients, four being associated with proteins and one with low molecular weight species.

The absence of bicarbonate in their buffer system [7] and the high aluminium content of the eluting buffer might be responsible for the different observation.

In conclusion, a significant amount of serum aluminium is ultrafiltrable, both in normal subjects and in patients with chronic renal failure. Transferrin is the main aluminium binding protein. The nature and significance of the ultrafiltrable and protein-bound aluminium in health and in patients with chronic renal failure need further study.

Acknowledgments

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References

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Open Discussion

VAN HERWEGHAM (Brussels) Do you have any information on the possible competition between iron and aluminium for transferrin and for desferrioxamine?

RAHMAN That is a very interesting question. We have no data on this particular point. It is however important as it might explain the anaemia associated with dialysis dementia, but this is hypothetical.

KERR (Chairman) I would like to comment that data presented at the Ankara meeting suggests that transferrin has about four orders of magnitude tighter binding for iron than for aluminium.
BRANCACCIO (Milan) Do you believe that patients affected by aluminium induced encephalopathy have different protein binding of aluminium in comparison to other patients on chronic dialysis?

RAHMAN We have not found any difference. There is one study showing that in patients with dialysis dementia most of the aluminium is bound to a low molecular weight protein of about 10,000 daltons. In addition the plasma concentration of this protein is higher in dementia patients than others.

KERR There are a number of papers indicating that aluminium appears to be bound to protein.

CANNATA Have you done any study on transferrin arising from the gastrointestinal mucosa?

RAHMAN I think that iron and aluminium shares the same transport mechanism in the intestinal wall. The competition between these two substances for absorption may explain some of your findings.