Aluminum Distribution in Serum Following Hemodialysis

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ABSTRACT

Aluminum is a toxic metal and has been implicated as the causative agent in the dialysis encephalopathy syndrome. In the study reported here, the transfer of aluminum to the blood from dialysis fluid during hemodialysis was demonstrated even when deionized water was used in the preparation of the dialysate. Studies were carried out on the binding of aluminum to serum proteins and other constituents in patients on long-term hemodialysis. Five major aluminum peaks were observed on chromatographic separation; four were associated with proteins and one large peak was probably associated with a low molecular weight species. The size of this latter peak was enhanced by increasing the aluminum content of the eluting buffer. It is postulated that this low molecular weight species might be the neurotoxic form of aluminum.

Introduction

Aluminum toxicity has, in recent years, been implicated in the pathogenesis of a number of clinical disorders in patients with chronic renal failure on long-term intermittent hemodialysis treatment.1,2,3,11 The predominant disorders have been those involving either bone (the osteomalacic component of dialysis osteodystrophy) or brain (dialysis encephalopathy). Two potential sources for the increased tissue content of aluminum in patients on hemodialysis have been proposed: (1) intestinal absorption from aluminum containing phosphate-binding gels, and (2) across the dialysis membrane from aluminum in the water used to prepare the dialysate.5,6,10,11,13 Significant loads of aluminum appear to be transferred to the plasma compartment and into tissues during the hemodialysis procedure from dialysates containing only very small amounts of aluminum.6,10

The occurrence of the dialysis encephalopathy syndrome (DES) has diminished during the past two to three years and the

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present authors are not aware of any reports in the last year. The disappearance of DES appears to be directly associated with the use of either deionized or otherwise purified water in the preparation of the dialysate used in the hemodialysis procedure.

The present study was undertaken to investigate the serum aluminum concentrations in hemodialysis patients before and after the hemodialysis procedure using deionized water and to confirm the extent of aluminum loading occurring even at very low dialysate aluminum concentrations. A related study also was undertaken in hemodialysis patients to separate the various proteins and other serum species which potentially bind aluminum in an attempt to determine if one of these might play a role in the etiology of the neurotoxicity. Aluminum bound to high molecular weight proteins would be less likely to cross the blood-brain barrier than lower molecular weight aluminum species.

Methods

Tap water samples (approximately 200 ml) were collected in acid-washed polypropylene bottles at the University of Virginia Medical Center Renal Unit and at the affiliated Annex Renal Unit. The tap water samples were collected when the tap was first opened in the morning (0 minutes) and after the water had run continuously for 10 minutes. This procedure was used in order to determine the effect on the aluminum concentration of the water after standing in the pipes overnight. Deionized water samples were collected after the water had been turned on for 10 minutes and a dialysate sample was taken from the site at which the dialysate entered the dialysis equipment. The latter sample was considered to be representative of both the Medical Center and the Annex since the same chemicals were used for preparation of the dialysate in both locations.

Blood samples were drawn into polypropylene tubes from the hemodialysis patients by venipuncture and allowed to clot. The tubes were centrifuged, the serum was removed and stored at -20°C until assayed.

Serum samples and serum-based aluminum standards (1.85, 3.71, 5.56, and 7.42 µmol per L) were diluted 1:25 with a 2 percent Triton X-100 in 1 percent ultrapure nitric acid diluent and analyzed for aluminum using atomic absorption spectrometry with electrothermal atomization. Serum aluminum concentration was determined using a standard curve generated with the absorbance values of the serum-based aluminum standards.

The water samples were analyzed using aluminum standards (0.074, 0.148, 0.222, 0.297, and 0.371 µmol per L) made up in deionized water. A 1.0 ml aliquot of each standard and water sample was diluted with 1.0 ml of 2 percent Triton X-100 in 1 percent HNO₃ and analyzed with the same procedure used for serum samples.

The serum distribution of aluminum was studied under equilibrium conditions using a gel filtration technique which has been previously reported. A 1 × 90 cm water jacketed column maintained at 37° was packed with Sephacryl S-200 superfine. The column eluent contained, per liter, 140 mmol of sodium

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<tr>
<td>Water Aluminum Concentration</td>
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<td>Aluminum, µmol/L</td>
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<td>Hospital</td>
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(130 mmol of NaCl, 6.8 mmol of NaOH, and 3.0 mmol of NaN₃), 1.1 mmol of CaCl₂ • 2H₂O, 0.5 mmol of MgCl₂ • 6H₂O, 4.0 mol of KCl, and 10 mmol of 2-(tris (hydroxymethyl)-methyl] amino) ethanesulfonic acid (TES).† The pH was 7.40 at 37°C. This eluting solution was analyzed and the aluminum concentration found to be 0.41 µmol per L.

After 1.5 ml of serum was applied to the top of the gel bed, 60 fractions with a volume of approximately one ml were collected. The contents of each tube were analyzed for aluminum, total protein and albumin as described previously.9

Results

The results of the water and dialysate study are shown in table I. There was essentially no difference between the aluminum concentrations found in the 0 minute and the 10 minute water samples. Thus, there was no evidence that the pipes used in the hospital water system contributed to the aluminum content of the tap water. The concentration of aluminum found in the tap water was consistent with that reported by the Virginia Water Control Board.15 The large decrease in the water aluminum concentration after deionization emphasized the need for chronic renal failure patients to be dialyzed against a dialysate made from deionized water in order to prevent an increase in blood aluminum and its subsequent deposition in body tissues.

The increased concentration of aluminum in the dialysate was due to the presence of aluminum in the mixture used to make up the dialysate. The effect of the aluminum in the dialysate on the serum aluminum concentration of the hemodialysis patients was studied by analyzing serum obtained from 45 chronic renal failure patients pre- and post-dialysis. The pre-dialysis patients had a mean aluminum concentration of 3.08 ± 1.04 µmol per L with a range of 0.85 to 4.86 µmol per L. The serum aluminum content in the post-dialysis specimens was 3.67 ± 1.30 µmol per L with a range of 1.85 to 8.12 µmol per L. There is a statistically significant increase in the serum aluminum concentrations of these patients following the hemodialysis procedure (t-test, p < 0.001). The results reported here are consistent with those of Kaehny et al6 who reported increased serum aluminum concentrations after dialysis even when the dialysate aluminum concentration was as low as 0.18 µmol per L.

The typical elution profile for a patient on renal dialysis (figure 1) was similar to the pattern obtained in a normal subject.9 The first aluminum peak (Y) which eluted before peak A was not present in the serum from the normal volunteer.9 The aluminum in the new peak Y must be complexed in some manner which creates a species large enough to be excluded from the column. Such a complex might be with protein, lipoprotein, cholesterol, or triglycerides. The fractions containing the aluminum in this peak were analyzed for cholesterol and triglyceride in order to determine if any lipid material containing these compounds was present; however, none was detected. This observation could have been due to the relatively low sensitivity of the methods used. Also, no protein was detected in this portion of the elution profile; again, this could have been due to the sensitivity of the method. Since aluminum is capable of forming colloids, the aluminum may have been complexed in a colloidal species which would be excluded from the column and thus elute in the void volume. Neither the composition of the aluminum or the significance of the aluminum in peak Y is understood. However, this peak has been present in elution profile of four renal patients and has not been seen in the profile using serum from a normal volunteer.

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Figure 1. Elution profile of renal dialysis patient.

Figure 2. Elution profile with potassium-free buffer and ultra-violet detector.
Aluminum in peak A was associated with some high molecular weight proteins present in the early elution of protein peak 1. This peak has been previously shown to contain alpha-2-macroglobulin, IgM, haptoglobin, and some orosomucoid. The present study has not provided information as to whether all of these proteins, a single one, or an undetected protein provides the binding of aluminum.

The aluminum in peak B was eluted in association with the albumin in peak III and this amount of aluminum was considerably greater in dialysis patients than in a normal volunteer. The increased amount of aluminum associated with the albumin peak may have been due to the higher aluminum concentration in the serum of the dialysis patients. The two largest aluminum peaks were peaks C and D. The nature of these peaks was studied by repeating the gel filtration procedure using an eluent free of potassium and by monitoring the eluted protein with a UV detector. The column fractions were analyzed for potassium and aluminum, and the results are shown in figure 2. The elution pattern was similar to that seen in figure 1. The greater separation between the aluminum peaks C and D in figure 1 and figure 2 was due to the slower flow rate used in the latter run.

The aluminum in peak C was probably bound to small inorganic species, such as phosphate and bicarbonate which were reported to elute just before the potassium peak in a calcium binding study using the same gel filtration technique employed here. However, the aluminum also might have been associated with the protein material in this peak, as shown by the absorbance of 280 nm. The aluminum in peak D was associated with a group of proteins and/or small polypeptides, as indicated by peak IV. Using polyacryla-
mide gel electrophoresis, two proteins were found under this peak with apparent molecular weights of 60,000 and 80,000. These proteins must have appeared in the later column fractions because of retardation owing to some interaction with the Sephacryl S-200 gel. Since amylase is known to interact with these type gels, the fractions were analyzed for amylase activity, and none was found. At this time the identities of these two proteins are not known.

In order to investigate the effect of exposing plasma to aluminum and imitating hemodialysis with aluminum contaminated dialysate, the serum from the renal patient used in figure 1 was chromatographed with an eluting buffer containing approximately 3.34 μmol per L of aluminum. In this procedure, the serum constituents that bind aluminum would take up aluminum from the buffer and possibly mimic the plasma binding of the aluminum that crosses the dialysis membrane. The elution pattern obtained with the added aluminum is shown in figure 3 and revealed an increase in the aluminum in peak A and caused peaks C and D to become one broad peak. No increase in the amount of aluminum bound to albumin or the peak in the void volume (peak Y) was observed. These results are consistent with the view that albumin is easily saturated with aluminum and the excess aluminum must be bound to other serum constituents.

Discussion

Aluminum in the serum of patients on long-term, intermittent hemodialysis has been shown to bind to high molecular weight proteins, albumin, low molecular weight proteins and/or polypeptides and inorganic anions.12 Large increases in the serum aluminum concentration, such as those that occur during hemodialysis against a dialysate containing large quantities of aluminum, were found to bind mainly to the inorganic anions, such as phosphate, citrate, and lactate. These results are considered with the findings of Karlik et al7 who demonstrated that the strong binding of aluminum by phosphate and citrate would completely prevent the interaction of aluminum with deoxyribonucleic acid. Also, Womack and Colonick16 reported that the inhibition of brain hexokinase by aluminum was due to the aluminum complexing with Na-K-Atpase and that this inhibition could be reversed by citrate and phosphate. No evidence for the existence of free or unbound aluminum has been found in this study. Since peaks A, B, and D are associated with large proteins and peak C is mainly associated with inorganic anions, the aluminum species in peak C may be the one that crosses the blood-brain barrier. The increased aluminum in peak C may also account for the increased urinary output of aluminum seen when plasma aluminum levels are increased6,10 and the increased amount of ultrafiltrable aluminum obtained when plasma aluminum concentrations are more than 7.42 μmol per L.4

The present study clearly demonstrates that patients on long-term intermittent hemodialysis treatment have increased serum aluminum concentrations following hemodialysis. Whether or not the serum aluminum can cause an accumulation of aluminum in the brain which is sufficient to result in the appearance of DES after many years of hemodialysis is not known. Because of this unknown future for hemodialysis patients, it would be interesting to follow these patients closely to detect possible large increases in serum aluminum which could lead to the onset of neurologic symptoms.

References