Ultrafiltration Studies in Vitro of Serum Aluminum in Dialysis Patients after Deferoxamine Chelation Therapy

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Using gel filtration chromatography, we evaluated aluminum bound to albumin, transferrin, and other plasma proteins in the serum of patients on maintenance hemodialysis. The proportion of dialyzable aluminum, as determined by selective membrane ultrafiltration and flameless atomic absorption spectrometry, increased by more than fourfold on treatment with the metal chelator, deferoxamine. This ultrafiltration technique may prove useful for monitoring the proportion of aluminum mobilized during such therapy. 

Aluminum (Al) poisoning in patients with chronic renal failure who are on long-term maintenance hemodialysis is now recognized as contributing to the dialysis encephalopathy syndrome and to dialysis osteomalacia (1, 2).

Efforts to remove Al from serum by direct hemodialysis have generally been unsuccessful, because 80% or more of the metal ion is bound to serum proteins such as albumin (3) and transferrin (4). Earlier attempts to reverse Al-induced syndromes with chelating agents such as dimercaptosuccinates or penicillamine (5) were not successful. However, Al can be mobilized and eliminated by dialysis (6–9) when another chelating agent, deferoxamine (DFO), is used.

Here, we examine, in chronic-hemodialysis patients who were undergoing Al detoxification therapy, the effect of DFO on Al bound to serum proteins. The mobilization (i.e., the detachment and removal) of Al from serum proteins was demonstrated in vitro by ultrafiltration and by gel-filtration chromatography.

Materials and Methods

Materials

Albumin (Cohn Fraction V, human) was from Sigma Chemical Co., St. Louis, MO 63178. Deferoxamine was supplied as "Desferal" (Ciba-Geigy Canada Ltd., Dorval, Quebec H9S 1B1). Human transferrin was obtained from Calbiochem-Behring, La Jolla, CA 92037. Rabbit antisera to human transferrin (from Dako, Denmark) was obtained via the distributor, Cedarlane Laboratories, Hornby, Ontario LOP 1E0. Bio-Gel P-2 (400 mesh; exclusion limit for molecules, 1800 Da) was from Bio-Rad Laboratories, Richmond, CA 94804). An ultrafiltration apparatus, the Centrifree Micropartition Unit, and membrane filters with approximate relative molecular mass retention of particles >1000 Da (Model YMT) were from Amicon Corp., Danvers, MA 01923. Unless otherwise specified, all other chemicals and reagents were of reagent grade or better and were from Fisher Scientific Limited, Don Mills, Ontario M3A 1A9.

Samples

Blood was sampled into 10-mL red-top Vacutainer Tubes (Becton Dickinson Co., Rutherford, NJ 07070). Individuals who had not received aluminum-containing antacids served as control subjects; they included 10 healthy laboratory staff and 24 hospitalized patients with normal renal function. Serum aluminum concentrations were monitored in uremic patients who were undergoing hemodialysis therapy at this hospital. From this group, 20 with Al concentrations >75 µg/L were given DFO, administered intravenously over 2 h in 500 mL of 50 g/L dextrose solution at a dose of 100 mg/kg body weight (to a maximum of 6.0 g). Blood was sampled for Al determination before the DFO infusion (before the initial hemodialysis), after DFO infusion (following the second hemodialysis), and then 48 h after the DFO infusion. This cycle was repeated after approximately one week, according to the patient's regular dialysis schedule.

Procedures

Total serum Al was determined by flameless atomic absorption spectrometry (AAS) as previously described (10). Assay precision was monitored with "in-house" quality-control sera (11), for which the within-run and between-run CVs were ≤5% and ≤8%, respectively. Analytical recovery of Al added to serum (to 200 µg/L) ranged between 95 and 103% by the method of standard additions.

The Al content of serum ultrafiltrates (Al fraction) was determined by a method similar to that described above for total serum Al. Approximately 1 mL of whole serum was centrifuged (1500 × g, 15 min, 4 °C) in a Centrifuge Micropartition Unit. The clear, colorless fluid that passed through the membrane filter was collected in the lower chamber of the unit and used for analysis.

Serum Al samples were fractionated by gel-filtration chromatography on 2 × 65 cm columns of Bio-Gel P-2. The columns were equilibrated and samples eluted, 1-mL fractions being collected, at a rate of 36 mL/h. The eluent was a solution containing 120 mmol of NaCl, 6 mmol of NaOH, 3 mmol of NaNO3, 1 mmol of CaCl2, 0.5 mmol of MgCl2, 4 mmol of KCl, 40 mmol of NaHCO3, and 10 mmol of Tris (pH 7.4 at 18 °C) per litre.

The fractions were monitored at 280 nm and then total protein was determined by the Coomassie Brilliant Blue binding method of Bradford (12), with a protein assay kit (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont. L4X 2A9), and albumin by the bromocresol green dye-binding method (13). Transferrin was detected immunochemically in the column fraction by use of the Ouchterlony diffusion technique. Al was determined in these fractions by AAS as described above.

Deferoxamine (in de-ionized water) was measured at 212 nm in a Model DU-8 spectrophotometer (Beckman Instruments Inc., Fullerton, CA 92634). The maximum absorbance wavelength shifted to 204 nm when a complex between DFO and Al was formed (Figure 1). This wavelength was used to detect the complex in aqueous solutions.
DFO to these patients, the total Al in the serum increased by more than twofold (p < 0.001) while the proportion of AlUF increased by more than fourfold (p < 0.001).

Figure 2 shows histograms illustrating the proportion of AlUF and bound Al for two patients on a regimen of twice weekly hemodialysis and approximately weekly infusions of DFO. These two patients illustrate that, before DFO infusion, the proportion of AlUF is about the same (50%) in both patients. Immediately after the first DFO infusion, the proportion of AlUF increased to 87% and 76% for patients A and B, respectively. The absolute magnitude of AlUF is quite different in these two patients, probably reflecting differences in initial Al burden. Patient A showed less overall fluctuation in total serum Al concentration over three cycles, while patient B’s response was more typical of that after DFO infusions. In both, the proportion of AlUF remained relatively high (>75%) 48 h after DFO infusion.

Although the results vary in different patients, subsequent infusions with DFO maintained the proportion of AlUF at >60% in this study.

By gel-filtration chromatography we separated constituents in serum samples to which Al may be bound. An index compound, vitamin B12 (1357 Da), was eluted from the column near the void volume, but ahead of the DFO:Al (approximately 587 Da) complex (Figure 3). When Al, as AlCl3, was loaded onto the column with NaCl, the sodium was detected by flame photometry at fraction 349, but Al was not detected in any of the fractions. However, when the column was subsequently loaded with DFO, the Al could be detected in the early peak in association with the chelator (Figure 3).

Results

Healthy controls, and patients with normal renal function who are not consuming Al-containing products, have values for serum Al that are within the reference interval (10), and the proportion of ultrafiltrable Al (AlUF) is 14.5 and 16.2%, respectively (Table 1). Patients on hemodialysis who had chronically ingested Al-based antacid gels had a high baseline value for serum Al and AlUF, 19.7% (Table 1), as previously reported (9). After the initial administration of

![Graph](image)

**Table 1. Aluminum Ultrafiltration in Controls and Dialysis Patients**

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Mean ± SD</th>
<th>AlUF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>10</td>
<td>7.8 ± 5.8</td>
<td>14.5 ± 3</td>
</tr>
<tr>
<td>Patients with normal renal function</td>
<td>24</td>
<td>14.0 ± 5.6</td>
<td>16.2 ± 4</td>
</tr>
<tr>
<td>Renal-dialysis patients</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-DFO</td>
<td>140</td>
<td>± 62*</td>
<td>19.7 ± 7*</td>
</tr>
<tr>
<td>Post-DFO</td>
<td>311</td>
<td>± 117*</td>
<td>85.8 ± 9*</td>
</tr>
</tbody>
</table>

AlUF, proportion of serum Al ultrafiltered. n, no. subjects. *p < 0.001 as compared with controls. ** p < 0.01 as compared with pre-DFO values.
The elution profile of the Al fractions in this major peak suggests a molecular mass of less than 500 Da, allowing dialysis through membranes that retain molecules of mass greater than 1000 Da.

**Discussion**

This study shows, by ultrafiltration in vitro, that a relatively large proportion of Al in serum is bound to plasma constituents, both in subjects with normal renal function and in uremic patients. This agrees with current reports that approximately 80% of the Al in serum is protein bound (14, 15) and is not diffusible through dialysis membranes. By the use of gel filtration chromatography we found that Al co-elutes with albumin and transferrin (as well as other unidentified constituents) as reported by King et al. (16) and Trapp (4). Trapp demonstrated by in vivo studies with $^{67}$Ga that Al ion is bound to one of two specific sites on serum transferrin. The binding of Al to transferrin is in direct competition with that of iron, which suggests a causative role for Al in the development of microcytic anemia that is not ascribable to iron deficiency (17), but further studies are required to see if this is so.

Since the introduction, in 1979, of DFO to remove Al from a patient with severe dialysis encephalopathy (6), this iron-chelating agent has been successfully used to treat cases of Al intoxication (7–9). Recent studies by Day et al. (18) suggest that DFO forms a six-coordinate 1:1 complex with the three available electrons of Al. In aqueous solution, this DFO-Al complex shows a characteristic maximum at 204 nm, as compared with 212 nm for DFO alone (Figure 1). In serum samples or ultrafiltrates of serum, it was not possible to measure DFO or its complex by spectrophotometry because of the high ultraviolet absorbance of the matrix at these wavelengths (Figure 1).

The ability of DFO to chelate Al and displace it from protein binding sites is clearly demonstrated by the shift in the gel filtration pattern of uremic patient sera before and after chelation therapy with DFO (Figure 4). The increase in total serum Al concentration after DFO infusion in several of the patients in this study, as illustrated in Figure 2B, is consistent with reports that DFO mobilizes Al in vivo from tissue and bone deposits (6, 8, 9, 19).
During hemodialysis, the DFO:Al complex, which has an $M_r$ of about 587, is able to pass through the dialysis membrane into the dialysis fluid under a suitable diffusion gradient. By the use of selective membrane filters (molecular mass cutoff about 1000 Da) in the Amicon ultrafiltration apparatus, under centrifugal pressure, we were able to assess the proportion of Al available for removal by hemodialysis. For example, a patient's Al$_{UF}$ before DFO treatment was 13.2%, which increased to 92.8% after DFO infusion and 48 h later was 84.9%. Another area in which the monitoring of the total Al and the Al$_{UF}$ was useful is in the magnitude of the response to DFO therapy. As illustrated in Figure 2B, a marked increase in Al$_{UF}$ was obtained in the initial infusion, which was reduced by about half in a subsequent DFO treatment. Because the Al burden differs in different patients, as also does the degree of Al mobilization with the chelator, the ultrafiltration technique can provide useful information on the status of Al$_{UF}$ for removal by hemodialysis.

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References