Treatment of Aluminum Overload Using a Cartridge With Immobilized Desferrioxamine

Sidney Anthone, Clara M. Ambrus, Romesh Kohli, Inkee Min, Roland Anthone, Agnes Stadler, Istvan Vladutiu

S. Anthone, C.M. Ambrus, R. Kohli, I. Min, R. Anthone, A. Stadler, I. Stadler, A. Vladutiu, Buffalo General Hospital, SUNY at Buffalo, Buffalo, NY


ABSTRACT

Intravenous desferrioxamine (DFO) is the method commonly used to treat aluminum toxicity. This laboratory has developed a hollow fiber device with immobilized DFO, an “Aluminum DFO-HP” (DFO-HP), for the purpose of removing aluminum without the chelator (DFO) entering the blood. With Food and Drug Administration approval, a polysulfone DFO-HP, placed in the extracorporeal circuit in series with the patient’s customary dialyzer, was tested for its safety and ability to remove aluminum in patients with ESRD who had aluminum overload. During treatment with this device, no toxic reactions, side effects, or hematologic or clinical laboratory changes were seen other than those associated with dialysis. Average aluminum clearance with the DFO-HP device was 25.3 mL/min with a range of 7.2 to 52.4 mL/min, whereas aluminum clearance with the F-60 polysulfone high-flux dialyzer was 8.4 mL/min. Aluminum clearance of the cuprophane dialyzers in series with the DFO-HP was negligible. The amount of aluminum removed over a 2-h treatment with DFO-HP ranged from 94 to 628 μg, which corresponded to 32 to 199% of the initial aluminum in the circulation before that particular treatment. The excess 99% was provided from aluminum released from tissue sites into the circulation throughout the duration of the treatment. The amount of aluminum removed decreased over time, so the efficiency and safety of the DFO-HP device, the time presently needed for aluminum depletion using intravenous DFO will be greatly shortened and the potential toxicity of intravenous DFO will be eliminated.

Key Words: Immobilized desferrioxamine, DFO-HP device, aluminum overload, aluminum removal

Presently, there are about 150,000 patients in the United States who have been diagnosed with ESRD and are being treated by some method of dialysis (1). It has been estimated that 30% of these patients have high tissue aluminum levels caused either by high aluminum content of the dialyzing solution and/or the ingestion of aluminum-containing compounds for the control of phosphate absorption. Furthermore, elevated aluminum levels correlated with the number of years patients spent on dialysis (2,3). A survey of 164 patients dialyzed at two hemodialysis centers in Buffalo, NY, revealed that 72% had serum aluminum levels twice normal. As illustrated in Figure 1, 17% had critical levels of aluminum of 100 to 200 μg/L or more, levels that would necessitate some type of treatment to prevent potentially serious toxicity (4–10).

Desferrioxamine (DFO) intravenously has been the most widely used therapy by the nephrologists to reduce high blood aluminum levels. DFO mobilizes aluminum from tissue stores. The DFO-aluminum complex is slowly filtered through the dialyzer membrane during hemodialysis. To obtain effective reversal of the aluminum overload, prolonged courses of treatment for up to 8 to 12 months were found to be necessary. The relatively poor removal of aluminum by the dialyzer, along with the slow release of the aluminum from the tissues, accounts for this protracted treatment course (11–13). Furthermore, treatment with parental DFO is not without dangers. There have been many reports alluding to the toxic effects of DFO itself: visual and auditory neurotoxicity, thrombocytopenia, yersinia sepsis, and mucormycosis infection have all been reported (14–18).

In order to avoid the potential toxicity of parenteral DFO and yet effectively remove aluminum, a device with immobilized DFO (DFO-HP) has been developed for the extracorporeal removal of aluminum. This chelator device is a high-flux polysulfone F-60 hollow fiber dialyzer (Fresenius AG, Bad Homburg, Germany) in which just 0.3 g of DFO has been immobilized. Each hollow fiber is made up of a capillary membrane at the lumen with a backing of a relatively thick macroporous substance that imparts mechanical strength to the membrane and serves as a support for the chelating resin. The DFO is deposited in this macroporous layer (Figure 2). As blood flows through the device, free aluminum and that bound to low-molecular-weight proteins filter through the membrane pores (30,000 kDa cutoff size) and are chelated by the immobilized DFO. The aluminum is removed from the circulation without the DFO or DFO-aluminum complex ever entering the circulation.
Immobilized DFO for Aluminum Removal

Figure 1. Aluminum levels (in micrograms per liter) of 164 hemodialysis patients: 72% of the patients had aluminum levels twice normal, and 17% had aluminum levels in the critical range of 100 μg/L or above, necessitating some type of treatment to prevent serious toxicity.

Figure 2. Schematic diagram of the cross-section of a single polysulfone hollow fiber. The chelator is immobilized into the macroporous layer behind the membrane of the lumen. The cutoff size of the membrane pores is 30,000 d.

The initial in vitro study of this new DFO-HP device has been reported (19-21) and revealed the rapid and effective clearance of both aluminum and iron. The device had also been applied in an extracorporeal circuit to large animals (dogs and monkeys) without causing physiologic side effects or any significant changes in biochemical or hematologic parameters (20,22).

An Investigational Device Exemption (IDE) was granted by the Food and Drug Administration for the evaluation of the safety and efficiency of this DFO-HP device in removing aluminum from dialysis patients with aluminum overload. This study was approved by the Institutional Review Board (IRB) of SUNY at Buffalo, School of Medicine and Biomedical Sciences, and the IRB of the Buffalo General Hospital.

PATIENT SELECTION AND TREATMENT PROTOCOL

Six ESRD patients being treated by hemodialysis were entered into this study. Each patient had initially a high serum aluminum level and had considerable elevation of this level after a DFO challenge (2 g of DFO i.v.) (Table 1). Each patient was treated once a week for 4 wk during the first 2 h of the first dialysis session of the week.

MATERIALS AND METHODS

The DFO-HP devices (Hemex, Inc. Buffalo, NY) were inserted into the dialysis circuit in series preceding the patient's usual dialyzer, and between the two, a Pall Extracorporeal Blood Filter LPE-1440 (Pall Corp. Glen Cove, NY) was inserted. The remainder of the extracorporeal circuit contained the usual monitoring devices and filter chambers used during the conventional dialysis procedure (Figure 3).

Blood samples were collected simultaneously at the blood sampling sites before and after the DFO-HP and after the dialyzer at 15, 30, 60, 90, and 120 min and were analyzed for aluminum content. The first two samples, being 15 min apart, were adjusted because of different initial blood flow rates and then figured as a 30-min value. Aluminum clearance and removal were calculated from the average of the four analyzed samples. Aluminum plasma clearance (Cl) was calculated by the formula:

\[ \text{Cl} = \frac{Q_b \times (C_b - C_a)}{C_b} \times (100 - \text{Hct}) \]  

where \( Q_b \) = blood flow, \( C_b \) = Al level before DFO-HP, \( C_a \) = Al level after DFO-HP, and \( (100 - \text{Hct}) \) = the adjustment made to calculate plasma volume. Clearance values were converted to micrograms of aluminum removed by the formula:

\[ \frac{\text{Cl} \times C_b \times 120}{1000} \]

Aluminum removed from the blood was also calculated by a direct method (23): \( Q_A = C \times F \times T \) where \( C \) = average difference in aluminum concentration before and after the DFO-HP, \( F \) = average plasma flow rate, and \( T \) = duration of treatment. Aluminum released was estimated as \( Q_A = V_p (C_a - C_b) + Q_{AI} \), where \( V_p \) is the total plasma volume and \( C_a \) and \( C_b \) are the concentrations of aluminum in the plasma at the end and the beginning of treatment, respectively.

Aluminum levels in serum samples were determined by atomic absorption spectrometry with a Perkin-Elmer Model 1100B spectrometer (Perkin-Elmer Corp., Norwalk, CT) (24,25) with a graphite furnace. DFO levels were measured by HPLC analysis (26,27). Levels of complement factors C3a and C5a were determined by a radioimmune assay per-

TABLE 1. Serum aluminum level before and after DFO challenge

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pre-DFO Challenge</th>
<th>Post-DFO Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>157</td>
<td>492</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>256</td>
</tr>
<tr>
<td>3</td>
<td>103</td>
<td>219</td>
</tr>
<tr>
<td>4</td>
<td>92</td>
<td>528</td>
</tr>
<tr>
<td>5</td>
<td>123</td>
<td>766</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>259</td>
</tr>
</tbody>
</table>
formed with a commercial assay system (Amersham International, Amersham, United Kingdom).

For the identification of serum protein fractions that carry aluminum, serum samples were separated by sieving chromatography with a column with Sephadex G-150 medium (Pharmacia, Biotech Inc., Piscataway, NJ) and a low-pressure gel permeation chromatography (GPC) system. The elutions were pooled according to specific molecular weight fractions. The protein content of the fraction pools was detected by absorption at 280 nm with a Pharmacia flow cell system; aluminum in the same pools was measured by atomic absorption spectrometry. Molecular weight standards used as controls were similarly subjected to gel filtration chromatography.

All reagents were tested for aluminum content as an aqueous solution. Laboratory equipment was washed with aluminum-free nitric acid rinsed with purified Type I water before usage and was used only if the discarded water was found to be metal free.

RESULTS

The average plasma clearance of the DFO-HP during the four consecutive runs for each patient is shown in Table 2. The spread between 7.2 and 52.4 mL/min for clearances is probably a reflection of the availability of diffusible aluminum, whether bound to low-molecular-weight proteins or released from proteins with a molecular weight above the cutoff size of the membrane pores.

The total amount of aluminum removed and released during the four treatments of 2 h each is shown in Table 3. The sudden removal of aluminum from the blood by the DFO-HP devices stimulated aluminum release from the tissues. The amount released is frequently more than the amount removed.

Aluminum clearance by the high-flux F-60 polysulfone dialyzer without the chelating agent was measured to be 8.4 mL/min. The aluminum clearance of the cuprophan dialyzers that were in series with the DFO-HP-devices revealed negligible if any clearance. Aluminum concentration was occasionally higher in the postdialyzer samples compared with predialyzer values, probably as the result of the hemoconcentra-

**TABLE 2. Aluminum clearances of the DFO-HP-devices during the first 2 h when applied in series with the conventional dialyzers**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Initial A1 (µg/L)</th>
<th>Avg. (mL/min) per treatment</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td></td>
<td>15.0</td>
<td>28.7</td>
<td>23.5</td>
<td>23.3</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td></td>
<td>32.6</td>
<td>25.6</td>
<td>32.1</td>
<td>28.0</td>
</tr>
<tr>
<td>3</td>
<td>111</td>
<td></td>
<td>17.7</td>
<td>17.9</td>
<td>16.9</td>
<td>18.6</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td></td>
<td>52.4</td>
<td>25.2</td>
<td>20.0</td>
<td>7.2</td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td></td>
<td>32.4</td>
<td>26.1</td>
<td>24.0</td>
<td>13.7</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td></td>
<td>24.6</td>
<td>20.6</td>
<td>44.5</td>
<td>37.3</td>
</tr>
</tbody>
</table>

* Each value represents the average aluminum clearance of five determinations taken during the 2-h application of the DFO-HP devices.
TABLE 3. Aluminum removal from plasma and release from tissues into the blood during 2 h of treatment with DFO-HP devices

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Plasma (μg/L)</th>
<th>Total μg in Plasma</th>
<th>Removed from Blood</th>
<th>Released into Blood</th>
<th>Total Aluminum (4 x 2 h Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>93</td>
<td>290</td>
<td>1,096</td>
<td>1,313</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>284</td>
<td>1,855</td>
<td>2,161</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>111</td>
<td>371</td>
<td>896</td>
<td>933</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>315</td>
<td>1,228</td>
<td>931</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>81</td>
<td>300</td>
<td>1,092</td>
<td>917</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>292</td>
<td>726</td>
<td>784</td>
<td></td>
</tr>
</tbody>
</table>

a Plasma volume calculated taking 5-L average blood volume and correcting for the hematocrit of the patient.
b Estimated as: \( Q_{A1} = C \times F \times T \). See text for explanation.
c Estimated as: \( Q_{i} = V \times C_{i} \times C_{d} + Q_{A1} \). See text for explanation.

Aluminum removal from plasma and release from tissues into the blood during 2 h of treatment with DFO-HP devices. The results obtained with the pretreatment and posttreatment samples are shown in Figure 5. Aluminum is noticeably present in all four protein fractions. The highest amount is found in the <30 kd pool that can easily filter through the membrane pores. This is also the pool that loses most of the aluminum (two-thirds) during the 2-h treatment. Proteins in the 30- to 60-kd molecular-weight pool lose only half of their aluminum content during treatment. Because these proteins cannot cross the fiber pores, we must assume that the missing aluminum was released from the protein into the blood and was subsequently chelated by the immobilized DFO. Proteins in the higher molecular weight range also carried aluminum, but the changes in their aluminum content during treatment with the DFO-HP were insignificant.

DFO stability within the DFO-HP was determined by measuring the DFO in blood samples removed before and after the 2 h of treatment. It is evident from the data that during the treatment the level of serum DFO either decreased or remained the same, but never became higher (Table 4).

In order to identify the source of DFO in the pretreatment serum of these patients, DFO levels were measured over a 40-day period in the serum of a hemodialysis patient after receiving a single challenge dose of DFO (2 g i.v.). We found that serum DFO levels fell gradually below a level of no detection only by the 39th day (Figure 6). Because all six patients in the study received their first treatment in less than 40 days (13 to 26 days) after the DFO challenge, the DFO...
Figure 5. Percent change in aluminum distribution among the various serum proteins during the treatment of a dialysis patient with DFO-HP (A1-H-P treatment). The largest amount of aluminum removed was from the smallest molecular weight (MW) fractions, which freely diffuse through the membrane pores. Aluminum lost from the proteins in the 30- to 60-kd pool was presumed to have been released from proteins that carried aluminum in an unstable binding.

**TABLE 4. Plasma DFO\(^\text{a}\) levels in dialysis patients before and after treatment with DFO-HP-devices**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Treatment No.</th>
<th>FO Plasma ((\mu g/mL))</th>
<th>Ratio a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>12</td>
<td>1.02</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>12</td>
<td>1.02</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>16</td>
<td>0.62</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>26</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>25</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>22</td>
<td>0.93</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>30</td>
<td>0.66</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>16</td>
<td>1.02</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>8</td>
<td>0.62</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>12</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>15</td>
<td>0.94</td>
</tr>
</tbody>
</table>

\(^\text{a}\) Measured with HPLC as ferroxamine (FO) calculated from area under the curve by the instrument.

present in the pretreatment samples can be considered the residual of the DFO injected at the time of the DFO challenge test.

During treatment with the DFO-HP-devices, no toxic reactions, side effects, or hematologic or clinical laboratory changes were seen other than those associated with dialysis. Levels of complement factors C\(_{3a}\) and C\(_{5a}\) showed only minor fluctuations that could be attributed to the patient's own dialyzer used during the chelating treatment. There were no significant changes seen during the 2-h treatment or at 4 h at the end of the conventional dialysis treatment. There were no clinical side effects observed by the clinicians or nurses during the treatments.

**DISCUSSION**

The treatment of aluminum overload with DFO-HP-devices is based on a different principle from the treatment with i.v. DFO. The DFO immobilized in the device removes aluminum from the circulating blood and keeps it in the device, without DFO ever entering the blood. The removal of aluminum from the circulation initiates aluminum release from the tissues soon after treatment begins. In turn, some of the released aluminum is also removed. This is evident from data in Table 3, showing that, frequently, the amount of aluminum removed from the blood was more than the amount of aluminum initially present in the blood. This excess aluminum was clearly provided from tissue deposits that released aluminum into the circulation.

The changes in blood aluminum level may also cause aluminum release from proteins with unstable aluminum binding (Figure 5). Aluminum bound to the high-molecular-weight proteins such as immunoglob-
uln M, immunoglobulin G, and transferrin will not filter through the membrane pores. The aluminum content of these protein pools did not change during the 2-h treatment of the patient with DFO-HP. Transferrin has been reported to the major aluminum carrier protein in human serum (28,29). On the other hand, aluminum present in the <30-kd fraction diffuses through the membrane pores, whether free or bound to small-molecular-weight proteins (30,31). The greatest decrease in aluminum was observed in this fraction. The 30- to 60-kd molecular-weight protein fraction lost about half of its aluminum content. It is possible that aluminum in this fraction pool is carried by albumin with an unstable binding that releases the aluminum when disequilibrium occurs. Such released aluminum is either captured by small-molecular-weight carrier proteins or diffuses through the membrane pores in a "free" form. In either case, the aluminum is removed from the circulation and is chelated inside the DFO-HP.

The application of the chelating device, DFO-HP, to the dialysis patients for 2 h of treatment was found to be safe. As blood flows through the hollow fibers of the DFO-HP, aluminum and aluminum bound to low-molecular-weight proteins were retained by the immobilized DFO. In addition, biochemical and hemolologic changes were only those that could be attributed to the patients' dialyzer that was in series with the DFO-HP. This would be in contrast to hemoperfusion and filter devices, which remove the DFO-aluminum complex while blood flows through the meshwork of the devices (32-34).

It appears, that the DFO-HP-devices provide patients with aluminum overload with a safe chelation procedure without the toxicity of DFO. It would be expected that the inclusion of these devices in the hemodialysis circuit during every dialysis treatment, for the entire duration of each treatment, would increase the efficiency of aluminum removal from the circulation and would shorten the time presently needed for aluminum depletion.

ACKNOWLEDGMENTS

Supported in part by a Grant R44 HL37821 from the National Institutes of Health, Public Health Service. We thank Rosemary Markus, MSN, for her help as monitor of the study. The assistance of the staff of the Hemodialysis Center at the Buffalo General Hospital is greatly acknowledged. For untiring effort, we thank Dianne Feind, MT/MRT, Catherine Kavai, Ellen Kenney, BS/MT, and Ellen Barnhart, SA.

REFERENCES