Aluminium removal with the double chamber technique: paired filtration-dialysis (PFD)

José L. Fernández-Martín, Walter Douthat, Susana Barreto, Alejandra Canteros, Gonzalo Acuña and Jorge B. Cannata Andía

Bone and Mineral Research Unit, Instituto Reina Sofía de Investigación, Hospital Central de Asturias, Universidad de Oviedo, Spain

Abstract Several dialysis techniques have been used to improve aluminium removal. So far there are no data available using paired filtration-dialysis (PFD). In this study, we evaluated the aluminium removed by PFD in two phases. Bovine plasma with known concentrations of aluminium and desferrioxamine was used in both experiments. In phase I, the aluminium removal was investigated using the PFD system (single pass) in its usual configuration, modifying the order of the convective and diffusive processes, dialysis with high permeability membranes and dialysis with low permeability membranes. During the second phase, the experiment lasted longer using recirculation, and the PFD was compared with conventional dialysis using high permeability membranes. Changes in the PFD configuration did not alter the aluminium removal; the efficiency of PFD for aluminium removal was very close to that of dialysis with high permeability membranes and much greater than with low permeability membranes. The aluminium is removed mainly in the first part of the dialysis. Aluminium mobilization using the double chamber technique (PFD) was efficient and might be of value for those patients with aluminium overload who needs high defferptive techniques and are unable to tolerate high-flux techniques.

Key words: aluminium clearance; aluminium transfer; desferrioxamine; highly permeable membranes; paired filtration-dialysis

Introduction

Prevention of aluminium exposure is the first and most important step in the treatment of aluminium overload [1]. However, prevention is not always sufficient and, on occasion, a technique which allows us to eliminate the already accumulated aluminium with minimum risk to the patient [2] must be added.

During haemodialysis, the elimination of aluminium is limited due to the fact that serum aluminium is bound mainly to plasma proteins of high molecular weight [3–5], in particular transferrin. Some years ago, despite this limitation, many patients had large elevations in serum aluminium. Nowadays, the lower serum aluminium observed in dialysis patients makes its elimination more difficult, because the ultrafiltratable gradient of aluminium is very low [6]. Due to this fact, most patients with aluminium overload need to increase the amount of ultrafiltratable aluminium using desferrioxamine. This drug acts by mobilizing aluminium in the tissues [7] and blood proteins [8] in such a way that, 44 h after its administration, high blood levels of ultrafiltratable aluminium susceptible to elimination through dialysis are achieved. Once the ultrafiltratable aluminium gradient increases, it becomes necessary to use adequate dialysis techniques which allow the maximum elimination of the aluminium–desferrioxamine complex formed. The use of high permeability membranes [9] and the combination of conventional dialysis and active charcoal cartridges have proven to be more efficient than low permeability membranes in the elimination of aluminium [10–12].

Paired filtration-dialysis (PFD) is a dialysis technique proposed to solve some of the problems involved in haemodiafiltration, such as interference in the transfer of the diffusive and convective mass, and the decrease in efficiency throughout the dialysis session, attempting to minimize the risk of back-filtration [13]. Schematically, this is a double chamber system in which the convective process takes places separately from the diffusive process, thanks to the use of a haemofilter placed in-series with a dialyser [14]. Volume restoration occurs through the infusion of a substitute liquid between both chambers (Figure 1).

The objective of this study was to evaluate the removal of aluminium by PFD, in its usual configuration, and modifying the order of the convective and diffusive processes, using as a clinical standard of
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Fig. 1. Phase I: scheme used in the single step PFD (UF, ultrafiltration; D, dialysis).

The removal of aluminium was investigated in four different situations: (a) PFD in its usual configuration (filtration with polysulphone and later dialysis with hemophan) (Figure 1); (b) PFD reversing the order of the process (first dialysis and then filtration); (c) dialysis only with a high permeability membrane (polysulphone); and (d) dialysis only with a low permeability membrane (hemophan).

In (a) and (b) (normal PFD and reverse order), polysulphone membranes of 0.70 m² (Spiraflo HFT06) and hemophan membranes of 1.18 m² (Spiraflo NT1208H) were used, as they are the surfaces currently most used with this technique. In the individual evaluation of high and low permeability membranes, the surfaces studied were two of the most currently used in dialysis patients (polysulphone: 1.00 m² Spiraflo HFT10 and hemophan: 1.18 m² Spiraflo NT1208H).

Six studies on each situation (a–d) were performed; in three we programmed a weight loss of 0.5 kg/session, and in the other three we increased the weight lost to 4 kg/session in order to study the effect on aluminium removal of the negative pressure applied in the dialyser. The restoration of aluminium and desferrioxamine kept the experimental conditions constant, making a correct interpretation of the results possible.

In each experiment, aluminium concentration was measured in plasma at inlet to the circuit and also in the ultrafiltrate or dialysate. The aluminium content of the dialysate fluid at the inlet of the dialyser (basal value) was to guarantee the highest possible percentage of ultrafiltratable aluminium (Al–desferrioxamine complex). Plasma was incubated overnight in order to allow the chelation of aluminium by desferrioxamine. To perform the experiment, we kept the plasma in a receptacle with a thermostatic bath to maintain the temperature at 37°C. Dialysis was done in a single pass system according to the scheme in Figure 1. At the end of each experiment, the plasma was re-used, restoring the volume lost in the first chamber (ultrafiltration) with a 0.9% sodium chloride solution. Aluminium and desferrioxamine losses were also restored.

The study was carried out in two clearly differentiated experimental phases, in which a Bellco Ultramatic B dialysis machine and a PFD WS monitor were used. The flow of blood (250 ml/min), ultrafiltration (50 ml/min), reinfusion liquid (50 ml/min) and dialysis liquid (500 ml/min) were maintained constant in both experiments (Figure 1). Weight loss was always programmed on the dialysis machine and not on the PFD monitor.

The experiments were done using bovine plasma, which was collected at the moment of the animal’s slaughter, anticoagulated with doses of 10 U/ml of sodium heparin and transferred to 500 ml bags after 4–6 h. Plasma was obtained later by centrifugation at 2000 r.p.m. for 20 min and, due to the greater protein concentration of the bovine plasma compared with the human plasma, it was diluted with a physiological solution to obtain a final protein concentration of 5.4 g/dl. The final pH of the diluted plasma was 5.8.

Phase I: experimental model with bovine plasma in single pass dialysis

We used 9 l of plasma with a very high aluminium concentration (200 µg/l) and an excess of desferrioxamine (≥ 100 mg/l) to guarantee the highest possible percentage of ultrafiltratable aluminium (Al–desferrioxamine complex). Plasma was incubated overnight in order to allow the chelation of aluminium by desferrioxamine. To perform the experiment, we kept the plasma in a receptacle with a thermostatic bath to maintain the temperature at 37°C. Dialysis was done in a single pass system according to the scheme in Figure 1. At the end of each experiment, the plasma was re-used, restoring the volume lost in the first chamber (ultrafiltration) with a 0.9% sodium chloride solution. Aluminium and desferrioxamine losses were also restored.

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In each experiment, aluminium concentration was measured in plasma at inlet to the circuit and also in the ultrafiltrate and/or dialysate. The aluminium content of the dialysis fluid at the inlet of the dialyser (basal value) was undetectable. Aluminium clearance was calculated using the following equation:

\[ \text{CL}_{\text{Al}} = \frac{C_D Q_{D0} + C_F Q_f}{C_{pi}} \]

where \( \text{CL}_{\text{Al}} \) = clearance of aluminium; \( C_{D0} \) = aluminium concentration in the dialysate; \( Q_{D0} \) = flow of dialysate; \( C_F \) = aluminium concentration in the ultrafiltrate (only PFD);
\[ Q_P = \text{flow of ultrafiltrate (only PFD)}; \text{ and } C_{Pi} = \text{plasma aluminium concentration at inlet}. \]

**Phase II: experimental model with bovine plasma and recirculation**

In this phase, 2 l of bovine plasma with 100 \( \mu g/l \) of aluminium and 10 mg/l of desferrioxamine were dialysed for 1 h; the aluminium and the desferrioxamine used were equivalent to the concentrations currently observed in the plasma of dialysis patients undergoing treatment for aluminium overload, 44 h after a dose of 30 mg/kg of desferrioxamine [15]. Two litres of bovine plasma was considered enough for 1 h of dialysis (equivalent to 8 l for a 4 h dialysis). In phase II, one of the main aims was to simulate the conditions found during 1 h in a real session of dialysis, and also to obtain the profile of aluminium removal during the session. Therefore, there was no restoration of aluminium and desferrioxamine, and fresh plasma was used for each experiment.

Two types of experiences were carried out: (i) PFD in its conventional configuration, with recirculation for 60 min, using the same kind of polysulphone membranes of 0.70 \( m^2 \) surface (UF, ultrafiltration) and hemophan membranes with a surface of 1.18 \( m^2 \) (D, dialysis) (Figure 2); and (ii) conventional dialysis, performed using polysulphone membranes with a surface of 1.09 \( m^2 \) (N = 4). The types of membranes used were the same as in phase I.

Plasma samples were obtained at 5, 10, 20, 30, 45 and 60 min pre- and post-dialyser. With the PFD technique, samples were obtained at the entrance and exit to the system. Aluminium clearance at each point was calculated according to the following equation:

\[ \text{CL Al} = \frac{(Q_P C_{Pi} - (Q_{Pi} - Q_F) C_{P0})}{Q_P} \]  

where \( \text{CL Al} = \text{clearance of aluminium}; Q_P = \text{plasma flow at inlet}; Q_F = \text{ultrafiltration flow}; C_{Pi} = \text{plasma aluminium concentration at inlet}; C_{P0} = \text{plasma aluminium concentration at outlet}. \]

An overall clearance of aluminium was calculated using the area under the clearance–time curve for each individual experiment.

Equation 2 calculates the aluminium clearance based on the changes in plasma aluminium. In addition, we also measured the aluminium clearance based on the amount of aluminium collected in the ultrafiltrate and/or in the dialysate (Equation 3). To do so, during all experiments the dialysate was collected in two clean plastic receptacles, following previously established and published methodology to avoid contamination [16]. The volume of the dialysate from the first 30 min of each experiment was collected in one of the receptacles (~15 l) and the volume from the second 30 min in the other. Later, after homogenizing the sample by shaking each receptacle, a sample for measuring aluminium concentration was obtained. In the PFD, the ultrafiltered fluid obtained in the convective sector was also collected, calculating the clearance with the following equation:

\[ \text{CL Al} = \frac{(C_D V_D + C_F V_F)}{C_{Pi}} \]  

where \( \text{CL Al} = \text{clearance of aluminium}; C_D = \text{aluminium concentration in the dialysate}; V_D = \text{volume of dialysate}; C_F = \text{aluminium concentration in the ultrafiltrate (only PFD)}; V_F = \text{volume of ultrafiltrate (only PFD)}; \text{ and } C_{Pi} = \text{plasma aluminium concentration at inlet}. \]

Aluminium measurements were done by graphite furnace atomic absorption spectrometry using a Z-3030 spectrometer, a HGA-600 graphite furnace and an AS-60 automatic sampler (Perkin Elmer), following the methodology already described [17]. All the reagents used were AR Merck® quality. The desferrioxamine used was Desferal (Ciba-Geigy).

The statistical comparisons were done by the ANOVA and the Student ‘t’ test for unpaired data, using the Systat for Macintosh program. The results are expressed as mean ± standard deviation.

![Fig. 2. Phase II: scheme used in recirculation (UF, ultrafiltration; D, dialysis).](image)
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Results

Phase I

No significant difference in aluminium clearance was observed in all cases when the programmed loss of weight of 0.5 kg/dialysis was increased to 4 kg/dialysis. Figure 3 shows the values of aluminium clearance obtained in the four conditions studied. The magnitude of the differences between a, b and c were minimal compared with the magnitude of the differences observed between any of these three clearances and hemophan (d). The latter was ~40% lower than the other three. No differences were observed in the removal of aluminium from plasma when the order of the PFD processes was reversed (b compared with a).

Phase II

The overall aluminium clearance, calculated by plasma difference (Equation 2, area under the clearance–time curve), was 25.2 ± 5.2 ml/min in PFD (i) and 28.9 ± 9.8 ml/min in dialysis with polysulphone (ii) (difference not significant). Aluminium clearance calculated by the aluminium removed in dialysis fluid (Equation 3) was 31.1 ± 2.4 ml/min with PFD (i) and 27.8 ± 4.7 with polysulphone (ii) (difference not significant). Note that the standard deviation using Equation 3 is much less than the standard deviation using Equation 2.

Figure 4 shows the aluminium clearance profile, which, in our study, could be performed only when we calculated the aluminium clearance based on the changes in plasma aluminium (six points, Equation 2). PFD and polysulphone profiles were similar, showing greater effectiveness at the beginning of the session but with large variations between measurements. The results of the aluminium clearance obtained by collecting the dialysate in the first and second 30 min of the experiment showed the same trend, but they did not allow us to establish a curve (only two points).

Discussion

PFD is a haemodiafiltration technique in which the two processes, convective and diffusive, are carried out separately. The combination of a haemofilter of polysulphone and a hemophan dialyser make it an efficient purification technique for small and medium sized molecules [18,19]. PFD assumes the characteristics of high efficiency and tolerance haemodiafiltration, with none of its negative effects, such as back-filtration and the need for a high blood flow. Several studies have evaluated aluminium clearance with different membranes [20] and dialysis techniques [12,21]; however, there are no data available on the behaviour of PFD with regard to the elimination of this metal.

Aluminium elimination during dialysis sessions is quite limited because of its binding to high molecular weight plasma proteins [22]. Desferrioxamine administration produces an increase in the level of total serum aluminium and its ultrafiltrable (dialysable) fraction due to the formation of the aluminium–desferrioxamine complex [23].

In our study (phase I), we investigated the aluminium clearance obtained with high plasma aluminium concentration reproducing a condition found in cases of heavy aluminium overload. In addition, the use of high aluminium concentrations guarantees a smaller error in our results, because the aluminium measurements loses part of its precision when the concentration of aluminium in the ultrafiltrate or dialysate is low. A high desferrioxamine concentration was also used to ensure that all the aluminium existing in the serum would complex with desferrioxamine (Al–desferrioxamine complex), allowing a maximum transfer of the metal.

PFD and dialysis with polysulphone showed higher efficiency than dialysis with hemophan (Figure 3). This is probably due to the greater hydraulic permeability.

Fig. 3. Clearance of aluminium found under the different conditions studied. (a) PFD (first ultrafiltration, second dialysis); (b) PFD (first dialysis, second ultrafiltration); (c) dialysis (polysulphone); (d) dialysis (hemophan). Results are shown as mean ± SD (*P < 0.001 with respect to a, b, c).

Fig. 4. Phase II. Aluminium clearance profile with PFD and conventional dialysis (HD). Each point shows mean ± SD.
of polysulphone, because the size of the holes in the membranes does not explain these differences. The molecular size of the Al–desferrioxamine complex is small enough to pass through the hole of both polysulphone and hemophan membranes, as shown in previous studies [20].

Others authors [18] have suggested that by changing the configuration of the PFD (first dialysis, second ultrafiltration), the removal of large molecules, such as β2-microglobulin, could be increased due to less competition with small molecules in the convective removal. In our study, changing the order of the filtration and dialysis procedures did not change the efficiency of PFD with respect to aluminium removal. If the diffusive process is done first, it reduces the possibilities of the convective process, and vice versa.

In phase II, we tried to make our study closer to the real situation found in dialysis patients with a smaller aluminium overload and receiving lower doses of desferrioxamine as treatment, i.e. serum concentrations of the drug close to 10 mg/l [15]. In these situations, after the infusion of desferrioxamine, aluminium in serum reached values in the range of 100 μg/l. Under such circumstances, there are no comparative studies of aluminium clearance [22].

Aluminium clearance is measured currently using two different techniques, one based on the analysis of plasma differences of aluminium and the other on the differences in aluminium concentration in the dialysate. Both methods have an important disadvantage which is that the differences in the aluminium concentration obtained in the pre- and post-samples are usually small and close to the detection limit of the aluminium measurement technique. In our study, in phase II, we measured the aluminium clearance using these two methods. The results were similar, although the calculations done using the differences in aluminium concentration in the dialysate showed more homogeneous results.

The findings obtained in phase II corroborated those obtained in the first phase, indicating that the mobilization of aluminium obtained with PFD is in the same range, although slightly lower than that obtained with high surface polysulphone. Nevertheless, strict comparisons between PFD and polysulphone results cannot be done in our study, because we did not use the same surfaces in the membranes. The maximum clearance obtained at any point for PFD in phase II was 56.2 ml/min (Figure 4, 10 min), whereas the clearance obtained for PFD in phase I was 99.6 ml/min. This difference could be explained by the different concentration of desferrioxamine used in both phases; in phase II the concentration of desferrioxamine was lower but closer to the real situation found in dialysis patients and, in these circumstances, we cannot guarantee that all aluminium forms the Al–desferrioxamine complex and thus some of the aluminium would remain bound to plasma proteins.

The profile of aluminium clearance showed a greater elimination in the first part of dialysis; similar results were observed in another previous preliminary study [2]. The rapid removal of the ultrafiltrable serum aluminium in the first hour abolished the aluminium gradient between the patient and the dialysis fluid. This fact explains the existing limitation on the mobilization of aluminium, which is based less on the permeability of the membranes and more on the tight binding of aluminium to plasma proteins, giving a minimal window for its removal by dialysis.

This fact should reinforce the concept that in the long-term management of aluminium overload, the chief efforts should be made in the application of strict norms for prevention of aluminium exposure [6].

In summary, conventional dialysis with polysulphone membranes and PFD in any configuration showed the best performance in aluminium removal, despite the fact that in the latter we used polysulphone membranes with lower surface area (30–40%). The use of PFD might be of value in removing aluminium in cases of aluminium overload in patients with intolerance to high-flux techniques. Further in vivo studies are required to confirm our findings.

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