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Removal of Aluminum from Chronic Dialysis Patients by Administration of Desferrioxamine and Dialysis

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Removal of aluminum (Al) from chronic dialysis (HD) patients by desferrioxamine (DFO), which was originally developed for elimination of excessive iron (Fe),[1] is worthy of note, not only because various clinical effects have been suggested,[2,3] but also because it is a test case of the combined therapy of drug administration with an extracorporeal procedure for removal of a deposited substance. However, the dynamic behavior of infused DFO, such as its *in vivo* interaction with Al and/or Fe, and the permeability of these substances across HD membranes, are not yet clarified. We therefore established a high-performance liquid chromatography (HPLC) system for measuring DFO in dialysate and plasma and also developed a leaky membrane with enhanced permeability to middle molecular substances such as a DFO–Al complex conceived. By making use of the method and membrane thus obtained, we performed quantitative *in vitro* and clinical evaluations of removal of deposited Al by the combined therapy.

Methods

Materials

Desferrioxamine B mesylate (Desferal; DFO) manufactured by Ciba-Geigy was used. Unless otherwise stated, DFO was intravenously administered to HD patients at a dose of 30 to 40 mg/kg body weight shortly after the completion of HD.

Two kinds of HD membranes were evaluated; one was a conventional cellulosic (cuprophane) membrane with a surface area of 1.0 m^2 and the other was the leaky poly(methyl methacrylate) (PMMA) membrane, Filtryzer BK (developed as TK-401 by Toray),[4] with the same surface area. The movements of various plasma proteins across these two membranes are listed in **Table 1** as sieving coefficients (the ratio of the concentration in ultrafiltrate to that in plasma).

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Analysis

Al in dialysate and plasma, and Fe in dialysate, were measured by conventional atomic absorption analysis, and Fe in plasma by the colorimetric method. For measuring DFO in dialysate and plasma, a new HPLC system was established.

During *in vitro* evaluation, ultraviolet (UV) spectroscopic, and fast atom bombardment–mass spectrometric (FAB–MS) (ZAB-HF mass spectrometer [VG]) methods were applied.

Subjects

Patients with end-stage renal failure undergoing HD at Kyoto First Red Cross Hospital were studied. As controls, two healthy volunteers also received DFO.

Data Analysis

Unless otherwise stated, the data were expressed as mean ± standard deviation (SD) and statistical analysis was performed by Student's *t* test.

Results

Measurement of DFO

By pretreating dialysate or plasma samples containing DFO with a cartridge SEP-PAK C_{18}, and then injecting the pretreated samples into an HPLC system using a reverse-phase C_8 column (YMC-PACK A-212) with ammonia-containing methanol as a mobile phase, concentrations of DFO in samples could be measured. The basic procedures of this method are listed in **Table 2.**

In Vitro *Interaction of DFO With Al*

UV spectroscopic analysis revealed a bathochromic shift of λ_{max} (wavelength of a maximum in the spectrum), and the broadening of the main peak, when an aqueous solution of DFO was mixed with that of aluminum chloride ($AlCl_3$). While the ϵ_{230} (molar extinction coefficient at 230 nm) of a DFO aqueous solution (0.01 to 0.1 mM) was 2,500, it was increased to 13,000 when $AlCl_3$ was added to the DFO solution.

On the other hand, FAB–MS analysis on the fraction obtained from passing the DFO–$AlCl_3$ mixture through HPLC

Table 1. Sieving Coefficients of Plasma Proteins Clinically Observed with BK (TK-401) and Cellulosic Membranes

Plasma Protein (mol. wt.)	Measurement Method	Sieving Coefficients (mean ± SD; n = 10) BK	Cellulosic Membrane
β_2-Microglobulin (11,800)	RIA	0.13 ± 0.06	~0
Retinol binding protein (21,000)	ID	0.019 ± 0.014	~0
α_2-HS glycoprotein (49,000)	ID	0.035 ± 0.024	~0
Prealbumin (55,000)	ID	0.029 ± 0.010	~0
Albumin (66,000)	ID	0.028 ± 0.006	~0
Transferrin (76,500)	TIA	0.023 ± 0.006	~0
IgG (150,000)	LIA	~0	~0
IgM (900,000)	LIA	~0	~0

RIA—Radioimmunoassay; ID—Immunodiffusion; TIA—Turbidimetric Immunoassay; LIA—Laser Immunoassay.

showed the basic cationic peak (M/e) at 585, which corresponded to (M-3H + Al) + 1 and confirmed the presence of a 1:1 complex between DFO and Al.

These data suggest that if only DFO and Al exist in an aqueous system, they interact and form a 1:1 complex.

Change in Plasma Al after DFO Administration

Twenty-eight patients undergoing HD three times a week for a prolonged period (more than 5 years, mean 9.5 years) (all taking aluminum gel), and 13 patients receiving HD at the same frequency for a shorter period (less than half a year, mean 3.7 months) (none taking aluminum gel), were monitored as to their plasma Al level before and after three consecutive DFO infusions (30 mg/kg each time). **Figure 1** depicts changes (mean ± SD) in plasma Al level in the long- and short-duration HD groups and in healthy controls. Markedly significant differences were noted between the long- and short-duration HD groups at 3, 5, 7, and 14 days after the first administration of DFO. Plasma Al in healthy controls presented markedly different absolute values and subsequent changes from those in HD patients.

Seventeen long-term HD patients were divided into two groups, one with and the other without bone pain and related complications. Changes in plasma Al observed in these two groups are depicted in **Figure 2.** As compared with the patients without bone pain, a significantly marked rise in plasma Al in those with bone pain was noted. **Figure 3** shows the

Table 2. HPLC Procedures

1. Prewashing of cartridge SEP-PAK C_{18} (MeOH → H_2O)
2. Injection of samples
3. Washing (H_2O)
4. Elution (NH_4OH-containing MeOH)
5. Concentration
6. Preparation of samples for HPLC
7. HPLC measurement

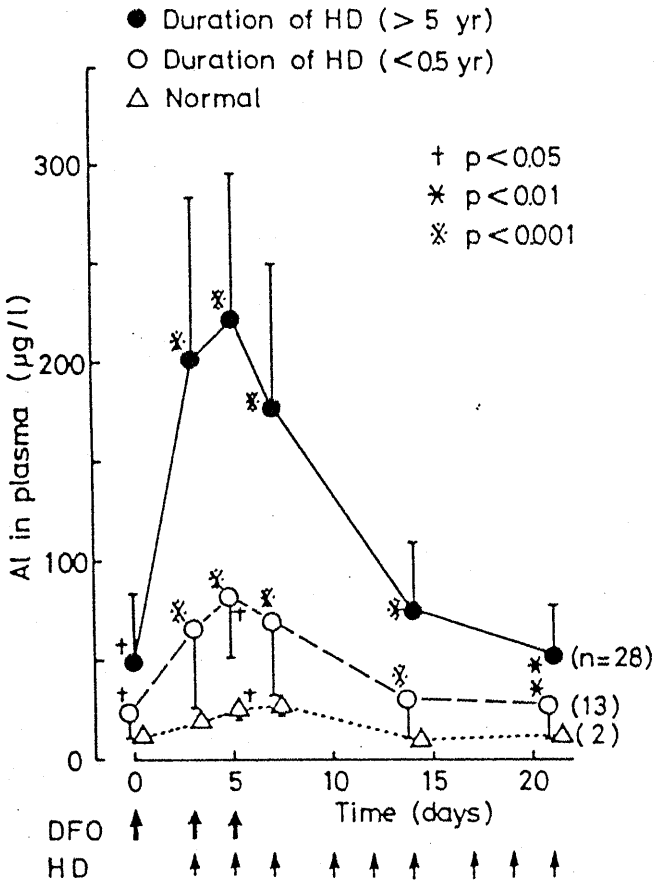

Figure 1. Changes (mean ± SD) of plasma Al levels in long- and short-duration HD patients and healthy controls. Lower arrows represent three consecutive DFO infusions for all subjects, and HD with conventional cellulosic membranes for HD patients.

comparison between patients given DFO for the first time and those who were already receiving DFO treatment for a mean period of 7.1 months. The former group tended to have higher Al levels, and subjective symptoms, such as bone pain, were alleviated in all the patients of the latter group.

Al Clearance

Three long-term (mean 5.6 years) and three short-term (7.2 months) HD patients were given DFO at a dose of 30 mg/kg at the end of HD, and plasma-basis clearance (C) of Al was determined by collecting the inlet and outlet blood samples at 1 and 4 h after the start of the next HD. By repeating this procedure, two kinds of HD membranes, BK and a cellulosic membrane, were compared. Blood flow rate (Q_b) was set at 150 or 200 ml/min, depending upon the tolerance of the patients, while dialysate flow rate (Q_d) was kept at 500 ml/min throughout the measurements. Although consistent C values of Al were not obtained when plasma Al levels were below 80 μg/l, a significant relationship between Al levels in plasma entering the dialyzer and plasma C values of Al was demonstrated in the range of plasma Al over about 80 μg/l as shown in **Figure 4.** The following regression equations of

C of Al as a function of plasma Al (x μg/l) were obtained: at a Q_b of 150 ml/min, C = 0.225x − 14.2 (r = 0.933, $P < 0.001$) and C = 0.133x − 11.7 (r = 0.660, $P < 0.025$); for the BK and cellulosic membrane, respectively (as depicted in **Figure 4**), and at a Q_b of 200 ml/min, C = 0.373x − 17.2 (r = 0.643, $P > 0.05$) for BK, and C = 0.151x − 4.1 (r = 0.487, $P > 0.1$) for the cellulosic membrane.

Membrane Permeability of Al and DFO

In order to confirm the difference in concentration dependent permeability of Al between the two membranes mentioned above, the membranes were replaced during HD from BK to the cellulosic membrane and *vice versa* in the next HD in the same four patients. Dose of DFO was adjusted to 40 mg/kg, and DFO in dialysate and plasma during HD also was monitored. Results obtained for four patients are individually depicted in **Figures 5, 6, and 7.** Although Al in dialysate tended to decrease during HD, as did the plasma Al, a rise of Al in dialysate was demonstrated only when the cellulose membrane was replaced with the BK. The change in DFO in dialysate well corresponded with that of Al, as shown in **Figure 7.** These data consistently suggest that BK is more permeable to both Al and DFO than is the cellulosic membrane.

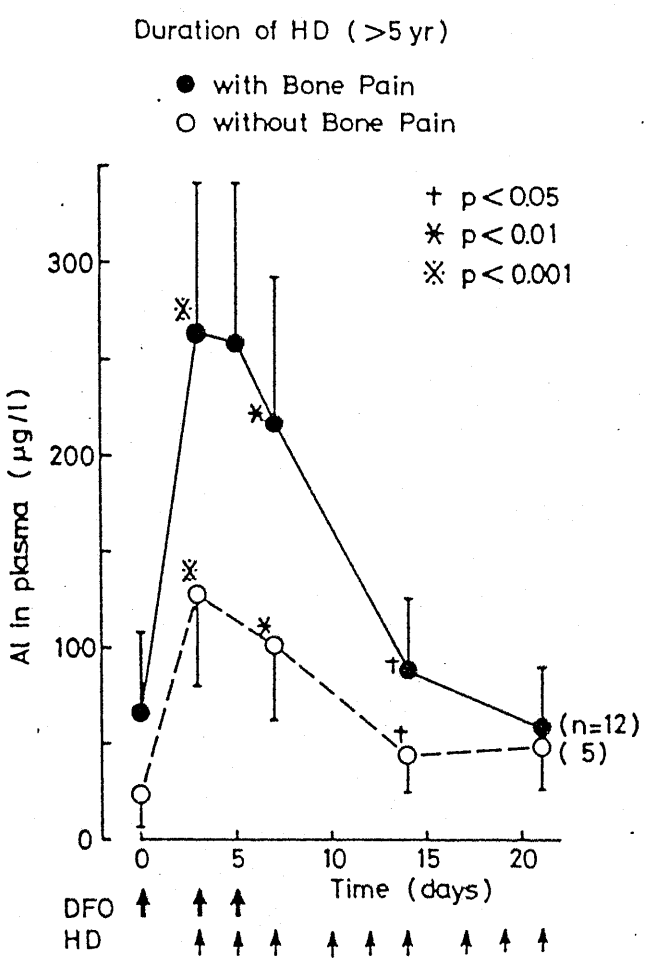

Figure 2. Changes (mean ± SD) of plasma Al levels in two groups of long-duration HD patients, one with and the other without bone pain and related complications. Lower arrows represent three consecutive DFO infusions and HD with conventional cellulosic membranes.

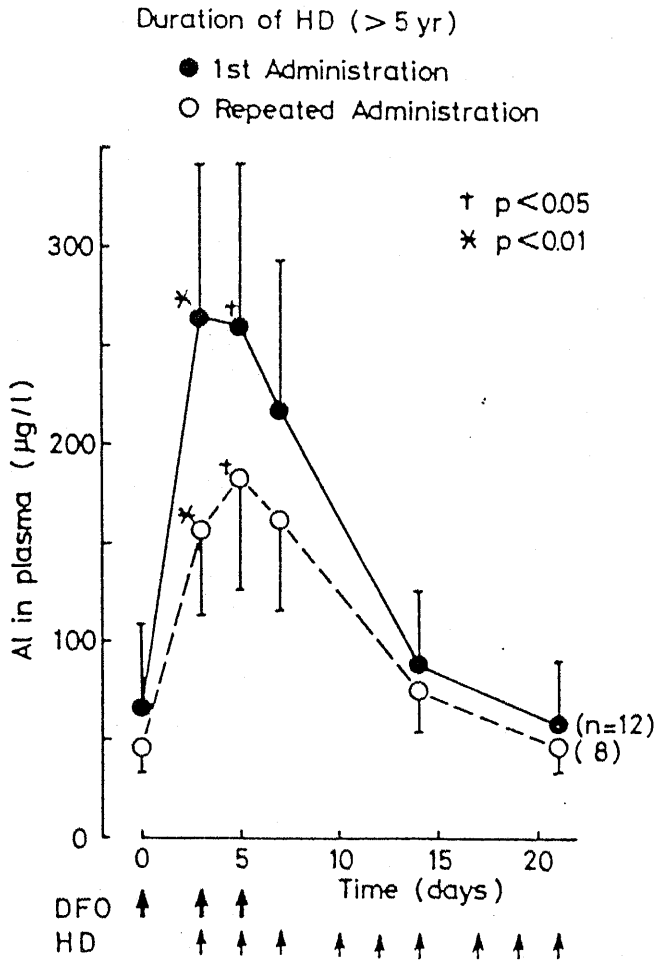

Figure 3. Changes (mean ± SD) of plasma Al levels in two groups of long-duration HD patients. Closed and open circles represent the data obtained on the first and repeated administration of DFO, respectively. Lower arrows represent three consecutive DFO infusions and HD with conventional cellulosic membranes.

In contrast to Al, plasma Fe remained unchanged during HD, and any significant difference in plasma Fe between inlet and outlet blood was not observed. Nevertheless, the diffusion of Fe into dialysate was quite similar to that of Al (decreasing during HD).

Dynamic Behavior of DFO in an In Vivo Setting

In **Figure 8** are shown the changes in plasma levels of DFO, Al, Fe, and ferritin after DFO infusion (40 mg/kg) from the end of one HD until the start of the next. The plasma level of DFO shortly after its infusion (mean 60 mg/l) corresponded to that estimated on the presumption that DFO is distributed in total body fluid (about 60% of body weight), followed by a rapid decrease. The level of DFO then stabilized, accom-

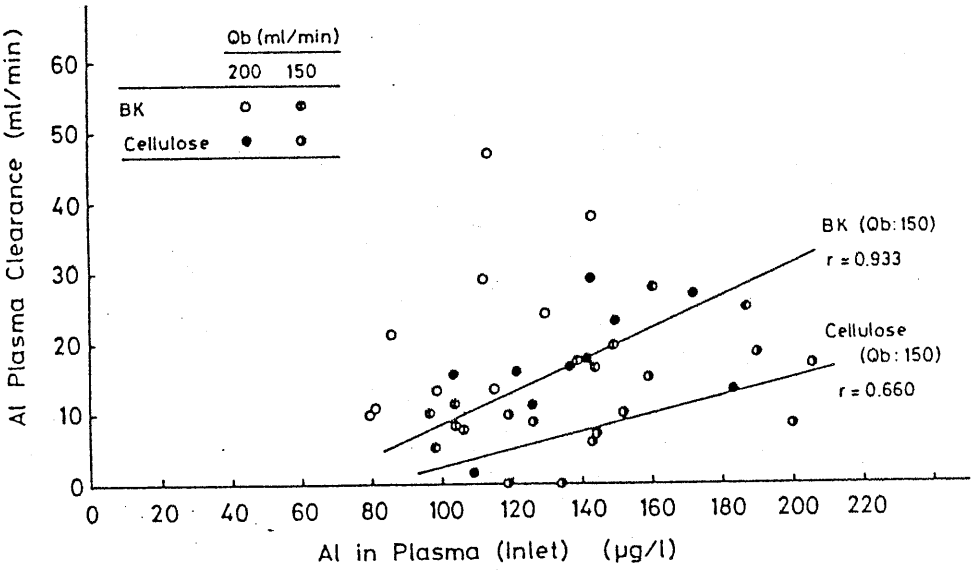

Figure 4. Relationship between Al levels in plasma entering the dialyzer and plasma clearance of Al with two kinds of membranes at two blood flow rates (Q_b). Regression lines at Q_b of 150 ml/min are depicted.

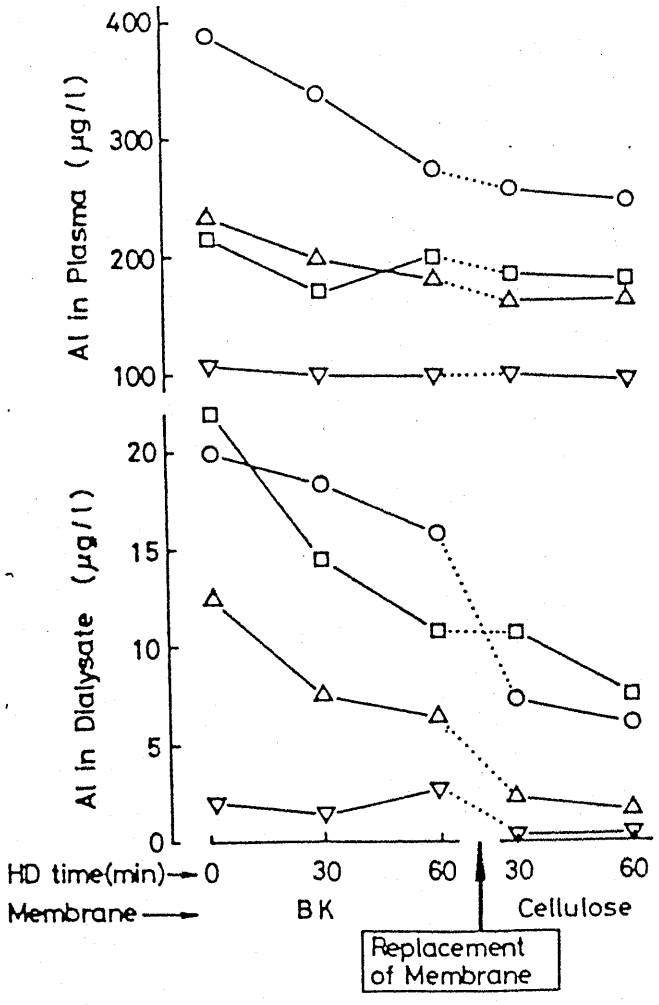

Figure 5. Changes of Al levels in plasma and dialysate during HD, where BK was replaced with the cellulosic membrane. Data of four patients are individually depicted with four different marks.

panied by an increase and then a leveling off of plasma Al. At the same time, plasma Fe remained unchanged except for an initial drop, and ferritin levels were quite stable, as were those of transferrin.

Discussion

DFO therapy has been suggested in HD patients for reduction of Al overload beause of its association with encephalopathy,[2] and more recently, bone disease[3] and anemia.[5] However, lack of fundamental data on the dynamic behavior or membrane permeability of Al and DFO has prevented us from optimizing DFO therapy for these patients.

In the present study, a new HPLC system for measuring DFO in dialysate and plasma was established, which allows the simultaneous monitoring of DFO and Al by adding DFO assay to the atomic absorption analysis for Al. In addition, a leaky membrane with significantly improved permeability to middle and large molecular substances (BK) was introduced to help clarify the membrane permeability of Al, for which conflicting views had been presented.[6,7]

At first, the clinical significance of the rise in plasma Al after DFO infusion was analyzed. As shown in **Figures 1, 2, and 3,** changes in Al level after DFO administration were greater in the long- *versus* the short-duration HD patients, in patients with bone pain *versus* those without it, and in patients receiving DFO therapy for the first time *versus* those undergoing repeated therapy. These observations suggest that the rise in plasma Al after DFO infusion can be used as a primary indication of Al accumulation, although strict examination, such as bone biopsy, should be performed for pinpointing the deposited Al.

As to the membrane permeability of Al, which had long been a controversial subject, this study revealed a concentration-dependent clearance of Al in the range of plasma Al over 80 μg/l, and a significant difference in Al clearance between the leaky membrane and the conventional one in that

range. The decreased clearance of plasma Al can be explained by assuming that part of plasma Al is bound to such nondialyzable plasma components as albumin or transferrin[7] and that the level of dialyzable Al increases with the increase in total plasma Al. In light of the data obtained in this study, it is further suggested that a significant amount of membrane-permeable and protein-unbound Al does not exist, unless total plasma Al exceeds about 80 μg/l and DFO increases the level of membrane-permeable Al. The greater permeability of Al with BK than cellulosic membrane was confirmed by replacement of the membranes during HD, which further revealed that BK is also more permeable to DFO than the cellulosic membrane. Judging from the data shown in Figures 5, 6, and 7, it can be assumed that Al and DFO jointly diffuse across HD membranes. However, Fe demonstrated different behavior from that of Al. For example, plasma Fe remained unchanged during HD, despite the comparable diffusion of Fe into dialysate. This fact may be interpreted as the participation of Fe in erythrocyte in its mass transfer across HD membranes.

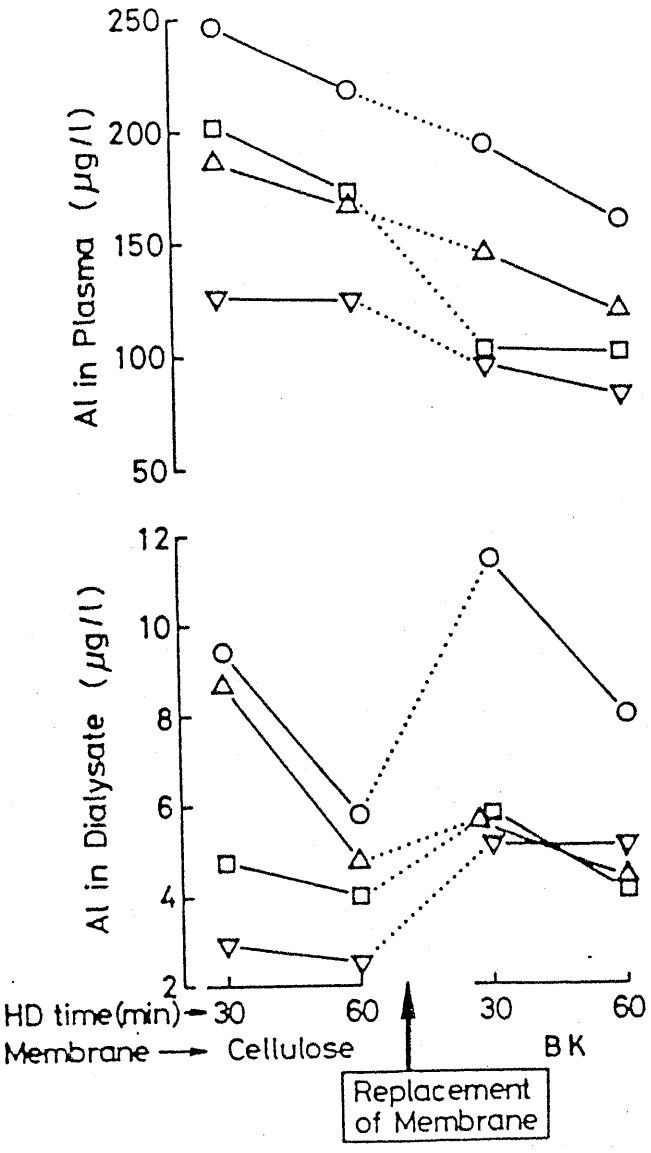

Figure 6. Changes of Al levels in plasma and dialysate during HD, where the cellulosic membrane was replaced with BK. Each mark represents data from individual patients.

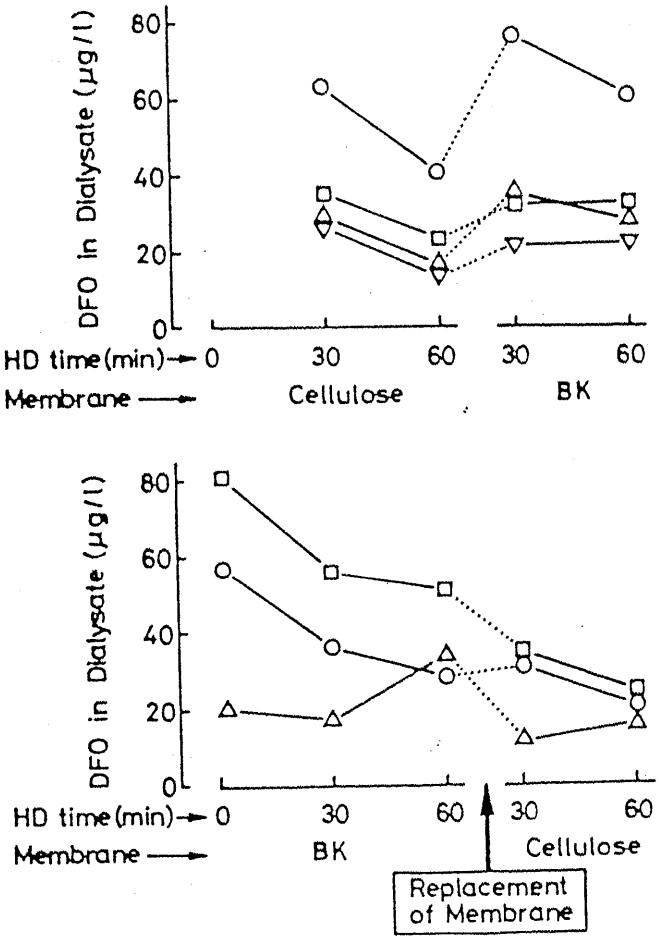

Figure 7. Changes of DFO levels in dialysate observed during HDs shown in Figures 5 and 6.

Just as the formation of a 1:1 complex between DFO and Fe reported by Keberle,[1] a 1:1 complex between DFO and Al in an aqueous solution was confirmed by FAB-MS analysis in this study. However, the ratios of DFO to Al or Fe detected in dialysate were inconsistent with the above *in vitro* observation. If DFO and Al form a 1:1 complex, the ratio of the concentration of DFO to that of Al should be 20:1. The ratio actually observed in dialysate was about 6:1. Furthermore, as shown in Figure 8, the ratio of the plasma level of DFO to that of Al in the stabilized phase was also about 6:1. Judging from our observation that DFO and Al apparently jointly permeate HD membranes, and that plasma DFO is stabilized with concomitantly stabilized Al, it is likely that a complex of DFO with Al also is formed *in vivo*. However, its coupling mode may be different from that *in vitro*.

Summary

In order to perform quantitative *in vitro* and clinical studies on the removal of Al by the combined therapy of DFO ad-

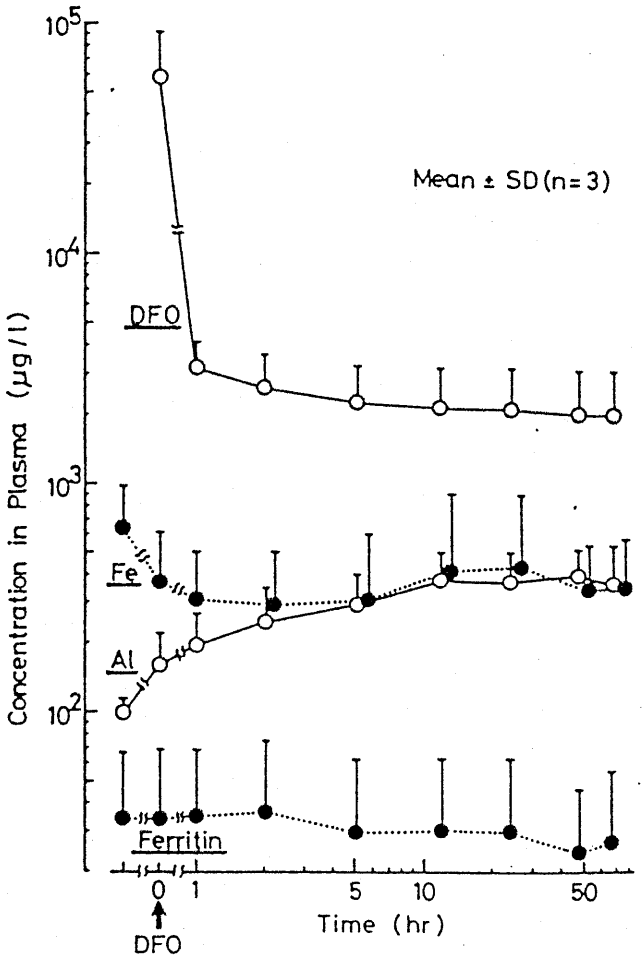

Figure 8. Changes (mean ± SD) in plasma levels of DFO, Al, Fe, and ferritin from DFO infusion to the start of next HD.

ministration and HD, an HPLC system for measuring DFO was established and a leaky membrane with enhanced permeability to middle molecular substances was developed. The DFO infusion caused rises in plasma Al levels (regarded as indicating the elution of accumulated Al) in patients undergoing HD. Plasma levels increased most in long-term patients and those with bone pain. Examination of Al clearances demonstrated that this increased plasma Al content passed through HD membranes at levels of more than 80 μg/l, and that the leaky membrane was more effective for removal of Al as well as DFO than the conventional one. Although a 1:1 complex between DFO and Al in an aqueous solution was confirmed, the formation of Al-rich complexes *in vivo* was suggested. It is concluded that Al-DFO complex formed *in vivo* can be effectively removed across the leaky membrane.

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