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Artificial Kidney and Dialysis

A new polymethylmethacrylate membrane for hemodialysis

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ABSTRACT: High molecular weight (MW) solutes are not removed during conventional hemodialysis (HD), and their accumulation is thought to play a role in some long-term HD complications (anemia, bone and joint pain, neuropathy, itching). The present trial was conducted to evaluate the removal capacity during in vivo *HD of a new polymethylmethacrylate (PMMA) membrane (Filtryzer BK-F, 1.3 m²) compared to conventional PMMA (BK-P, 1.6 m²) and to cellulose acetate (CA, 1.3 m²). BK-F dialyzers, with a pore size of 100 A° and 62% porosity, are designed to remove high MW substances. Ten stable anuric RDT patients (53 ± 13 years) were treated for one week with each membrane in a randomized sequence. Plasma concentrations of creatinine, BUN and beta$_2$-microglobulin (beta$_2$-M) were measured before (b) and after (a) HD to determine the reduction rate for these substances (%). Beta$_2$-M concentration after HD was corrected for changes in distribution volume. Samples of spent dialysate were collected after 3 minutes, 120 minutes and at the end of HD sessions, and appropriately treated and concentrated for HPLC analysis. The reduction rate for BUN and creatinine was similar for the 3 membranes. BK-F showed a higher beta$_2$-M reduction rate than BK-P ($p<0.005$) or CA ($p<0.0001$). HPLC analysis of dialysate showed prevalent peaks < 4 kilodaltons (kDa) throughout HD for BK-P and CA. Solutes > 10 kDa were infrequently detected. Peak profile during HD with BK-F was quite different, showing a predominant peak > 50 kDa which also included albumin. However, albumin loss significantly decreased after 120 minutes and at the end of dialysis compared with the 3-minute values, and was lower than that reported in CAPD patients. With BK-F a peak of MW > 500 kDa was also detected which previous studies indicated as a range characterized by the presence of erythropoiesis inhibitors. Use of the BK-F membrane in HD could afford satisfactory removal of high MW substances, thereby preventing or controlling some long-term HD complications such as anemia or beta$_2$-M amyloid formation. (Int J Artif Organs 1996; 19: 232-9)*

KEY WORDS: High performance liquid chromatography, Polymethylmethacrylate membrane, Beta$_2$-microglobulin, High molecular weight solutes, Hemodialysis

INTRODUCTION

Several high molecular weight (MW) solutes have been identified as potential uremic toxins. These include beta$_2$-microglobulin (beta$_2$-M) (1), parathyroid hormone (2), granulocyte inhibitory protein (3), degranulation-inhibiting protein (4) and furancarboxylic acid (5), although it is conceivable that other, as yet unknown, large uremic toxins remain to be identified. High MW toxins are not removed by hemodialysis (HD) with conventional membranes, and their accumulation may play a role in some long-term HD complications (6). To remove high MW solutes, highly permeable membranes and therapies including convective transport, such as hemofiltration and hemodiafiltration, have been proposed. However, the use of

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convenction-based treatments has been limited due to high cost and technical complexity.

The polymethylmethacrylate (PMMA) membrane is a synthetic membrane with a good permeability to solutes (7, 8) and an elevated biocompatibility profile (9, 10). This membrane was developed for the removal of beta$_2$-M, which is the major constituent of amyloid fibrils in HD-related amyloidosis (1). A long-term multicentre clinical study on the removal of beta$_2$-M by PMMA membrane (BK dialyzer) has demonstrated a significant decrease in both plasma beta$_2$-M levels (though they did not normalize) and amelioration of joint pains (8). The efficiency of conventional PMMA in removing beta$_2$-M is mainly by adsorption (11).

Recently, a new large-pore-size PMMA membrane (BK-F dialyzer) has been developed. The BK-F membrane has a pore size of 100 A° and 62% porosity, and has been designed for the removal of high MW solutes during HD. In the present study we evaluate the removal capacity of the new PMMA membrane compared to conventional PMMA and cellulose acetate membranes. Removal capacity during HD was assessed by both the reduction rate for small and large molecules and by analysis of the solutes present in spent dialysate by means of high performance liquid chromatography (HPLC). Each patient included in the study was dialyzed with each membrane in order to reduce individual variability and obtain comparable results.

PATIENTS AND METHODS

Patient population

Ten stable end-stage renal disease patients (7 males, 3 females) on chronic HD treatment for at least 6 months were enlisted in this study after giving their informed consent. The mean age of the patients was 53.4 ± 13.1 years (range 33-70). All patients were anuric, and their interdialytic weight gain was < 5% of body weight. None of the patients was known to have a malignant disease or monoclonal gammopathy, or to suffer from infections or severe cardiopathy. During the study period, no change was made in the medical therapy or dialysis schedule.

Study protocol

Three different membranes were tested in this study: cellulose acetate, conventional PMMA, and the new PMMA. Cellulose acetate (CA) dialyzer (FB 130T; Nipro) with a surface area of 1.3 square meters (m^2); the conventional PMMA membrane (BK-P; Toray) with a 1.6 m^2 surface area; the new PMMA dialyzer (BK-F; Toray) with a 1.3 m^2 surface area. All the dialyzers were "first use" and had been gamma ray sterilized.

The study covered 3 weeks' treatment, with 1 week's treatment on each dialyzer. The dialyzer sequence was randomized. Samples for analysis were collected during the second treatment at a 2-day interval. HD procedures were performed with a volumetric control device. The blood flow rate was 300 ml/min, dialysate flow rate was 500 ml/min and dialysis time was 240 minutes per session. The dialysate used during the study was identical for all treatments and contained sodium (138 mEq/liter), potassium (2 mEq/liter), calcium (3.5 mEq/liter), and bicarbonate (34 mEq/liter).

The procedures followed were in accord with the Helsinki Declaration.

Analytical procedures

For the determination of the reduction rate of blood urea nitrogen (BUN), creatinine and beta$_2$-M blood samples were taken immediately before and after the dialysis session. Heparin was used as the anticoagulant for plasma preparation, and separation from red blood cells was performed immediately. The plasma concentrations of BUN and creatinine were determined using standard laboratory techniques, whereas the plasma concentation of beta$_2$-M was determined by enzyme immunoassay (Enzygnost beta$_2$-microglobulin Behringwerke, Marburg, Germany). The reduction rate was calculated as follows: reduction rate, % = [(a-b/a] x 100, where a = plasma concentration before HD, and b = plasma concentration after HD. The beta$_2$-M concentation after HD was corrected for changes in distribution volume (12). The pre- and postdialysis BUN values were used with the Daugirdas formula (13) to calculate KT/V.

Samples of spent dialysate (10 liters) were taken at predetermined intervals (3 minutes, 120 minutes and end of dialysis). About 500 ml of dialysate was first

concentrated by ultrafiltration with YM-10000 (Amicon-Grace) at 2 ml (about 22 mg/ml) and subjected to HPLC analysis. Gel filtration experiments were performed at 25° C using a Protein Pak 300 SW (Waters) column (0.87 cm x 30 cm) connected to an HPLC system equipped with a rheodyne injector and a photodiode variable-wavelength detector (Kontron). Samples (200 µl) of ultrafiltrate were injected into the column, pre-equilibrated and eluted isocratically at a flow rate of 1 ml/min with 0.1 M potassium phosphate buffer (pH 6.5) containing 1 mM/EDTA. The eluate was monitored at 280 nm. The following proteins of known molecular mass were used as standards: ferritin (440 kilodaltons, kDa), bovine serum albumin (67 kDa), ovoalbumin (43 kDa), chymotripsinogen A (25 kDa), ribonuclease A (13.7 kDa). Peak profile by HPLC analysis was based on the retention time of solutes, the latter depending on the MW of the solutes. To compare and statistically analyze the removal capacity of the three dialyzers according to the solutes detected in the dialysate by HPLC, solutes were grouped into ten peaks of different MW (Tab. I). By means of the HPLC method the albumin loss in the dialysate (expressed as mg/10L) was identified and quantified. The total albumin elimination during the HD procedure was calculated using the mean concentration from dialysate at 3 min, 120 min and end of dialysis. For this analysis, we assumed that the dialysate flow remained constant at 500 ml/min.

Statistical analysis

Statistical analysis was performed using the statistical package JMP SAS and SPSS. Results are expressed as mean ± standard deviation (SD). To evaluate the normal distribution of age and sex of the study patients, the Shapiro-Wilk test and one-way variance analysis, were used respectively. The statistical comparison among the three membranes as regards the reduction rate of BUN, creatinine and $beta_2$-M was performed by Friedman's test for related samples. The same test was used to compare the KT/V calculated for the three membranes. The four parameters (reduction rate of BUN, creatinine, $beta_2$-M; KT/V) were also considered simultaneously and compared among dialyzers by means of discriminant analysis. The correlation between the above mentioned parameters was evaluated by R^2 index (determination coefficient R^2). To compare the removal capacity of the three dialyzers according to the peaks detected by HPLC analysis, for all patients we calculated the percentage of presence of a peak in the dialysate (presence of the event) during HD (three HPLC determinations) with each membrane. The percentage of presence of the event was compared among the three dialyzers by means of the Chi-square test (maximum presence for a dialyzer, 30 events=100%, when a peak was found in all ten patients in each of the three HPLC determinations: 10 patients x 3 times = 30 events). The time course of albumin loss during HD was evaluated by analyis of variance for repeated measures followed by a paired t-Test for significant differences.

The level of statistical significance was defined as $p<0.05$.

RESULTS

Study outcome

Age and sex of patients were normally distributed within the study group. No adverse clinical reactions were observed with any of the dialyzers used. All patients enlisted tolerated the therapeutic procedures well and completed the study with no drop-outs.

Reduction rate for BUN, creatinine and $beta_2$-microglobulin and KT/V

The reduction rate of BUN and plasma creatinine was not significantly different among the three dialyzers. The reduction rate (%) of BUN was 65 ± 6 for dialysis with CA, 66 ± 7 with BKP and 65 ± 7 with BKF; reduction rate (%) of plasma creatinine was 57 ± 7 (CA), 58 ± 8 (BKP) and 58 ± 7 (BKF). As far as the reduction rate of $beta_2$-M is concerned, statistically significant differences between the dialyzers were observed (Fig. 1). The greatest removal of $beta_2$-M was performed by BK-F (31 ± 8%) which proved to be significantly higher than the $beta_2$-M reduction rate of both BK-P (15 ± 14%, $p<0.005$) and CA (-1.6±12%, $p<0.0001$). In its turn, BK-P removed a significantly higher amount of $beta_2$-M than CA ($p<0.05$).

KT/V, calculated according to the Daugirdas formula (13), was not statistically different among the three

TABLE I - PERCENTAGE OF PRESENCE OF SOLUTES IN THE DIALYSATE

	CA	BK-P	BK-F	Significance
Peak 1 MW > 500 kDa	3.33%	3.33%	100%	BK-F vs BK-P and CA
Peak 2 MW 156-121 kDa	16.6%	10%	50%	BK-F vs BK-P and CA
Peak 3 MW 87-52 kDa	23.3%	33.3%	100%	BK-F vs BK-P and CA
Peak 4 MW 11.6 kDa	16.6%	23.3%	10%	NS
Peak 5 MW 10.7-7.7 kDa	23.3%	6.6%	42.5%	BK-F vs BK-P
Peak 6 MW 5.9-5 kDa	100%	96.6%	33.3%	BK-P and CA vs BK-F
Peak 7 MW 4.6 kDa	0	6.6%	26.6%	BK-F vs BK-P and CA
Peak 8 MW 4.2-3.9 kDa	0	0	16.6%	BK-F vs BK-P and CA
Peak 9 MW 3.6-3.3 kDa	100%	100%	100%	NS
Peak 10 MW <2.8 kDa	100%	100%	93.3%	NS

CA = cellulose acetate; BK-P=conventional PMMA; BK-F=new PMMA.
Significance is defined as $p<0.05$ by Chi-square test. NS = Not significant. For details, see section Patients and Methods

dialyzers tested: 1.09 ± 0.2 for CA, 1.08 ± 0.2 for BK-P, and 1.08 ± 0.2 for BK-F.

Discriminant analysis was used for "simultaneous" evaluation of the four parameters (reduction rate of BUN, creatinine and beta$_2$-M; KT/V) in order to further compare the dialytic efficiency of the three hemodialyzers. This analysis confirmed that the removal of beta$_2$-M (reduction rate) was the main parameter differentiating the three dialyzers, the highest value having been found with the BKF dialyzer (Wilks' Lambda = 0.41743, F=18.84, p=0.0001). A strong correlation was found between KT/V and creatinine reduction rate (r=0.93), BUN reduction rate and creatinine reduction rate (r=0.92), and between KT/V and BUN reduction rate (r=0.96).

HPLC analysis of dialysate

Analysis of spent dialysate by HPLC was performed at different time points (3 min, 120 min, end of dialysis) during HD sessions with the three hemodialyzers tested. HPLC analysis during dialysis with CA showed prevalent (as a percentage of relative area) peaks < 4 kDa of MW (Fig. 2). A small peak between 6.0 and 5.0 MW was however observed in all determinations. A similar chromatographic pattern was observed during dialysis with BK-P (Fig. 3). Peaks of MW higher than 10 kDa were infrequently found during HD with these dialyzers. The peak profile in the dialysate was quite different during dialysis with BK-F (Fig. 4). A prevalent peak > 50 kDa was observed for all patients at the different time points of analysis. This peak included albumin. Albumin loss in the dialysate was negligible with both CA and BKP dialyzers. Albumin loss, expressed as mg/10 L dialysate, was 346 ± 147 after 3 minutes of HD with BK-F, but significantly decreased after 120 minutes (253 ± 54, $p<0.05$) and at the end of dialysis (230 ± 80, $p<0.02$ vs 3 minutes; p=NS vs 120 minutes). The calculated total albumin loss during the HD procedure with BK-F was 3.3 grams.

Table I shows the results of the Chi-square test relating to comparison of the percentage of HPLC detection of a solute in the dialysate (presence of the event) during dialysis with CA, BK-P and BK-F. As reported, statistically significant differences between BK-F and both BK-P and CA were found for solutes up to a MW of 3.9 kDa, with the only exception of

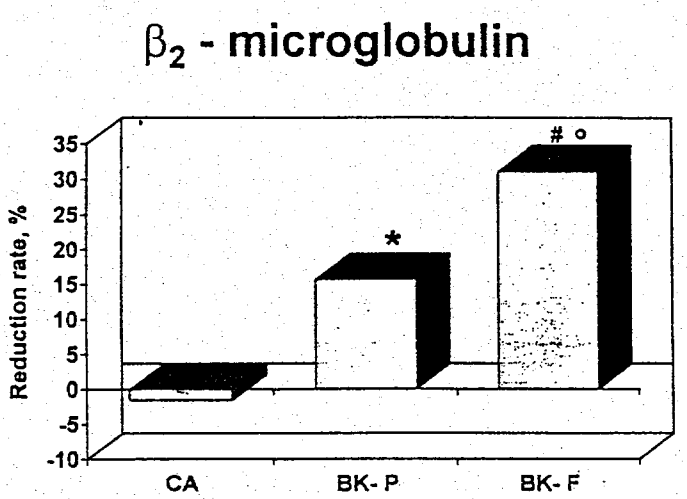

Fig. 1 - *Reduction rate of beta$_2$-microglobulin during dialysis with cellulose acetate (CA), conventional polymethylmethacrylate (BKP), and the new polymethylmethacrylate (BKF). Beta$_2$-microglobulin levels after dialyis were corrected for changes in distribution volume.*
** p<0.05 BKP vs CA; # p<0.005 BKF vs BKP; ° p<0.0001 BKF vs CA*

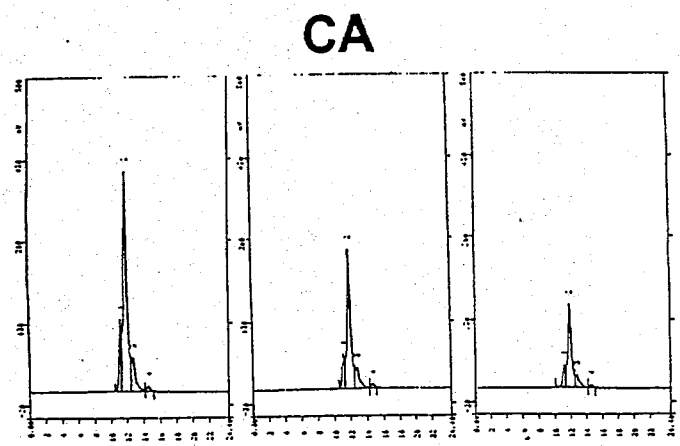

Fig. 2 - *HPLC analysis of dialysate at different time points during dialysis with cellulose acetate. The chromatograms (left=3 minutes; mid=120 minutes; right=end dialysis) are those from one representative patient out of ten. Molecular weight of peaks is as follows: peak 1, 5.8 kDa; peak 2, 3.06 kDa; peak 3 and peak 4, <2.0 kDa.*

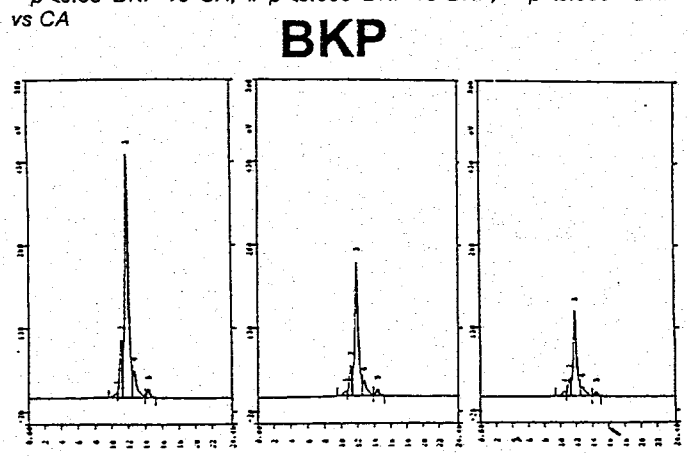

Fig. 3 - *HPLC analysis of dialysate at different time points (as in Fig. 2) during dialysis with conventional polymethylmethacrylate membrane (BKP). The chromatograms are those of the same patient as in Figure 2. Molecular weight of peaks is as follows: peak 1, 10 kDa; peak 2, 5.8 kDa; peak 3, 3.2 kDa; peak 4 and peak 5, <2.0 kDa.*

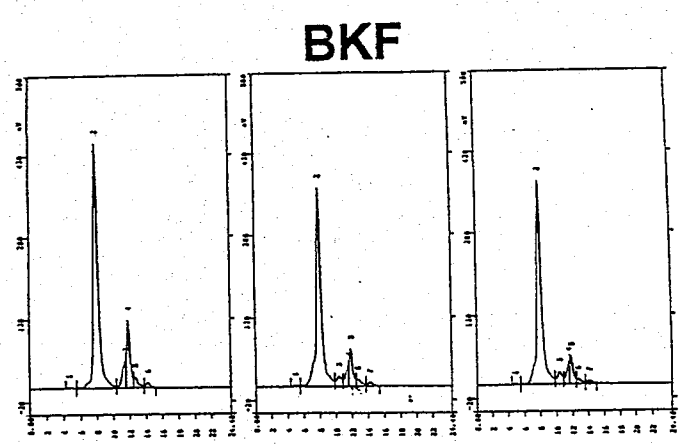

Fig. 4 - *HPLC analysis of dialysate at different time points (as in Fig. 2) during dialysis with the new polymethylmethacrylate membrane (BKF). The chromatograms are those of the same patient as in Figures 2 and 3. Molecular weight of peaks is as follows: peak 1, >500 kDa; peak 2, 70 kDa; peak 3, 4.2 kDa (3') and 10.7 kDa (120' and 240'); peak 4, 3.1 kDa (3') and 4.2 kDa (120' and 240'); peak 5, <2 kDa (3') and 3.1 kDa (120' and 240'); peak 6, <2 kDa (3') and 2.5 kDa (120' and 240'); peak 7 (at 120' and 240'), <2.0 kDa.*

peak 4 and peak 6 (Tab. I). For solutes of MW<3.4 kDa, no significant difference between the dialyzers was found.

DISCUSSION

The failure of dialysis to eliminate high MW solutes might be responsible for some complications (hematological, neurological, immunological; bone and joint disorders) frequently observed in chronic hemodialysis patients (6, 14). Since high MW solutes as well as highly protein-bound toxins are not removed by conventional HD, alternative dialytic strategies for the removal of these solutes should be considered (15). In previous studies, the PMMA membrane has demonstrated a satisfactory dialytic removal of solutes

including beta$_2$-M (7, 8, 16). To further enhance the dialytic efficiency of the dialyzer, a new large-pore-size PMMA membrane (BK-F dialyzer) has been developed which possesses a broader MW fractionation curve designed to allow the removal of higher MW solutes (17). We conducted a prospective cross-over trial evaluating the removal capacity during *in vivo* HD of the new PMMA membrane in comparison to conventional PMMA and cellulose acetate membranes. Removal during HD sessions (measured as the reduction rate) for small molecules such as urea and creatinine was similar for the three membranes. The beta$_2$-M reduction rate was, however, significantly greater with BK-F than with the other two membranes (Fig. 1). The accumulation of beta$_2$-M has generally been implicated in the genesis of amyloid disease which is frequently observed in long-term dialysis patients (1, 18-20). Since the major cause of increased plasma beta$_2$-M concentration in renal failure is the loss of renal function (21), it appears essential to try to remove as much beta$_2$-M as possible during dialysis (8). At least by adsorption, PMMA (BK membrane) can remove a greater amount of beta$_2$-M than other synthetic membranes (16). In a 2-years multicenter study the use of PMMA (BK membrane) showed a significant decrease in plasma beta$_2$-M levels and amelioration of joint pain (8). The results obtained in our study showing significantly higher beta$_2$-M removal by BK-F than by the other PMMA membrane (BK membrane) could be of clinical interest since the onset of beta$_2$-M amyloidosis may be delayed by vigorous efforts to lower the plasma beta$_2$-M concentration (20, 22). In this respect, as pointed out by previous results (8), it is advisable to use the BK-F membrane continuously right from an early phase of HD therapy.

The main difference in the removal capacity of the three hemodialyzers tested in this study was disclosed by the HPLC analysis of spent dialysate at different time points during the HD procedure. While during HD with both CA and BK-P membranes the chromatographic pattern was characterized essentially by solutes (peaks) <4 kDa of MW, dialysis with BK-F was associated with the detection of high MW solutes (>50 kDa) in spent dialysate. This high MW peak included albumin. Identification of the other solutes detected by HPLC in spent dialysate lies beyond the scope of this study. An important requirement in HD using highly permeable membranes is that albumin loss be limited, since this may result in catabolism, malnutrition, and an increase in total cholesterol and triglycerides as well as in nephrotic syndrome (23). On the other hand, beneficial effects on anemia (24) as well as bone and joint pain and uremic neuropathy (23) have been observed with membranes leaking 6-8 (24) and 7.5 (23) g/dialysis of albumin. Furthermore, the use of protein-leaking membranes may represent a way to remove highly protein-bound toxins which cannot be removed by conventional HD (15). An example of such a substance is furancarboxylic acid (CMPF), which inhibits erythropoiesis (25), drug-binding (26), hepatic glutathione S-transferase (27), and oxidative phosphorylation in mitochondria (5). CMPF, being highly albumin-bound (>95%), is not removed by conventional HD: however, despite loss of a considerable amount of albumin into the dialysate, CAPD can efficiently remove CMPF (25). This different removal pattern for CMPF may determine the lower CMPF levels in patients on CAPD than on HD (25, 28), and may partly explain the higher hemoglobin levels seen in CAPD patients (28).

For HD with BK-F, we calculated an albumin loss of 3.3 grams/dialysis (membrane adsorption of protein was not considered in this calculation), which is lower than that of CAPD and that of hemofiltration (14). Further, it should be noted that no significant change in the blood levels of albumin, IgG, IgA and IgM was found after an 11 month-period using BK-F membranes in HD patients (17). The albumin loss may be compensated by an increased protein content in the diet which can be achieved through dietary counseling for protein intake. We also observed that albumin loss significantly decreased during the course of HD in time. This finding may be explained by the formation of a secondary membrane (caused by deposition of different blood substances after blood contact with the dialysis membrane surface) as reported in previous studies with other dialyzers (14, 29). Therefore, as suggested (14), during HD with highly permeable membranes the albumin loss might be minimized by using a slow blood flow and a low transmembranous pressure during the starting phase of HD sessions until the secondary membrane is formed (formation time 20 minutes). In all chromatograms obtained during HD with the BK-F dialyzer we detected a small peak of MW >500 kDa (Fig. 4 and

Tab. I). Our results are in agreement with previous studies showing the presence in the dialysate of patients dialyzed with the BK-F membrane only (not with the BK-P membrane) of a fraction (called KR4-0) whose estimated MW was between 500 and 1000 kDa (17). The KR4-0 fraction has an inhibitory effect on the formation of mice bone marrow erythroid progenitor cells (CFU-E) (17). Inhibition of erythropoiesis, though not the only factor, is involved in the anemia of renal failure (30). Furthermore, the degree of inhibition of erythropoiesis (inhibition of CFU-E) may determine the severity of anemia in dialysis patients (31). Thus, use of the BK-F dialyzer might improve the anemic state of HD patients by removing the high MW inhibitor of erythropoiesis. Since KR4-0 seems to have an inhibitory effect on the late stages of erythropoiesis, which are mainly regulated by erythropoietin (EPO), use of the BK-F could also result in an improvement in the cost-benefit ratio of EPO therapy by reducing the necessary dosage of EPO to maintain the hematocrit target level. While only long-term prospective clinical studies can definitively clarify this issue and the other benefits resulting from the use of the BK-F membrane in HD, the preliminary results (32) do seem to support this assumption.

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