Protein-Leaking Membranes for Hemodialysis: A New Class of Membranes in Search of an Application?

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A new class of membranes that leak protein has been developed for hemodialysis. These membranes provide greater clearances of low molecular weight proteins and small protein-bound solutes than do conventional high-flux dialysis membranes but at the cost of some albumin loss into the dialysate. Protein-leaking membranes have been used in a small number of clinical trials. The results of these trials suggest that protein-leaking membranes improve anemia correction, decrease plasma total homocysteine concentrations, and reduce plasma concentrations of glycosylated and oxidized proteins. However, it is not clear yet that routine use of protein-leaking membranes is warranted. Specific uremic toxins that are removed by protein-leaking membranes but not conventional high-flux membranes have not been identified. It is also unclear whether protein-leaking membranes offer benefits beyond those obtained with conventional high-flux membranes used in convective therapies, such as hemofiltration and hemodiafiltration. Finally, the amount of albumin loss that can be tolerated by hemodialysis patients in a long-term therapy has yet to be determined. Protein-leaking membranes offer a new approach to improving outcomes in hemodialysis, but whether their benefits will outweigh their disadvantages will require more basic and clinical research.

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Uremia is characterized by retention of solutes over a wide molecular weight range (1), and new solutes are being added to the list every year. The toxicity of some low molecular weight substances, such as water and potassium ions, has been long established, and current dialysis prescriptions are able to maintain their levels in a quasiphysiologic range. In many instances, however, the relationship between retention of a particular solute and a specific toxicity is poorly defined.

Evidence has been accumulating steadily that low molecular weight proteins in the size range 5 to 35 kD are important uremic toxins that contribute to the high morbidity and mortality associated with chronic kidney disease. For example, the HEMO study demonstrated that increasing urea removal did not improve patient outcomes (2), whereas use of high-flux membranes, which allowed some modest removal of low molecular weight proteins, was associated with an improvement in cardiovascular outcomes (3). Exactly which low molecular weight proteins are important in uremia remains unclear. Most attention has been paid to β_2-microglobulin because of the high serum concentrations found in patients with ESRD and the formation of debilitating β_2-microglobulin amyloid deposits in bone, tendons, and joints (4). However, many other low molecular weight proteins with demonstrated or probable toxicity, such as complement factor D and leptin, are also retained in uremia (1). In addition, proteins may undergo posttranslational modification in the uremic environment (5,6), and this posttranslational modification may lead to toxicity even if the protein is present at an essentially normal concentration. For example, albumin modified by either glycosylation or oxidation can stimulate monocytes to produce reactive oxygen species that are thought to play a role in the oxidant stress associated with uremia (7).

Uremia is also characterized by the retention of protein-bound solutes that have an effective molecular size the same as that of their carrier protein. Many small protein-bound solutes are retained in chronic kidney disease (1), and some of these have been demonstrated to exert a toxic effect at the concentrations observed in ESRD. For example, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (240 Da), which is 98% bound to albumin, inhibits erythropoiesis (8), and p-cresol (108 Da) and indoxyl sulfate (251 Da), both of which are nearly 100% protein bound, inhibit endothelial proliferation and wound repair (9).

Identification of low molecular weight proteins and protein-bound solutes as uremic toxins, together with the failure of increased small solute removal to improve patient outcomes (2), has promoted interest in increasing the removal of larger solutes. High-flux membranes have been developed and are in widespread use, and there has been increased interest in alternative modes of renal replacement therapy, such as hemodiafiltration and hemofiltration, that enhance large molecule removal. The main focus of membrane development has been to sharpen the molecular weight cutoff of the membrane to maximize removal of low molecular weight proteins while minimizing the removal of albumin, which has been considered undesirable, as discussed later. Recently, some investigators

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have questioned the conventional wisdom that albumin loss is harmful to patients and have advocated the use of a new class of membranes that allow some passage of albumin and other similar-sized proteins as a means of increasing the removal of low molecular weight proteins and, possibly, unidentified toxic solutes in the 35- to 60-kD size range. This review summarizes the current status of this research, including what has been learned in the clinical application of these protein-leaking membranes in the chronic treatment of ESRD and the questions that remain to be answered before a decision can be made about their clinical utility.

Is Some Albumin Loss Acceptable?

By design, protein-leaking membranes allow passage of some albumin and other similar-sized proteins into the dialysate or filtrate. The conventional wisdom holds that this protein loss is potentially harmful. A low serum albumin concentration has been well documented to be a predictor of mortality in hemodialysis patients (10,11), and albumin loss through conventional high-flux membranes processed for reuse with bleach is associated with a decrease in serum albumin concentration (12,13). An argument can be made, however, that a modest loss of albumin does not pose a risk to most patients. Healthy individuals excrete in their urine >1.3 g/d albumin in the form of albumin fragments derived from processing of filtered albumin in the kidney (14). Furthermore, renal excretion of albumin may be more pronounced for modified forms of albumin (15). From this perspective, some loss of albumin into the dialysate or filtrate might be considered beneficial, given the presence of modified albumin in the serum of hemodialysis patients (5,6). In addition, Kaysen *et al.* (16) showed that a decrease in albumin synthesis, rather than increased albumin loss, is the principal cause of the low serum albumin in hypoalbuminemic hemodialysis patients. The decrease in albumin synthesis may be related to protein-calorie malnutrition or inflammation (17,18). The majority of hemodialysis patients are able to increase their rate of albumin synthesis and maintain a normal serum albumin concentration (17). That a modest loss of albumin into the dialysate or filtrate may not necessarily have an adverse impact on serum albumin concentrations is supported by observations in peritoneal dialysis patients. Caravaca *et al.* (19) observed a small, nonsignificant decrease in serum albumin concentration over a period of 24 mo in a group of 17 stable peritoneal dialysis patients despite a protein loss in the range of 5 to 7 g/d. Serum albumin concentration decreased in seven patients and remained unchanged in 10 patients. Protein loss in the effluent dialysate was greater in the patients with a stable serum albumin concentration than in those in whom serum albumin concentration decreased (7.44 ± 2.08 *versus* 5.14 ± 2.05 g/d) (19), presumably reflecting the role of serum albumin concentration as a driving force for both diffusive and convective solute removal. Taken together, these data suggest that a modest loss of albumin and similar-sized proteins into the dialysate or filtrate does not cause problems in well-nourished hemodialysis patients.

Membranes and Mechanisms of Solute Removal

Traditional low-flux hemodialysis membranes were restricted in permeability by the need to limit water flux in the absence of dialysis equipment that was capable of controlling water removal. The advent of volume control systems in the late 1980s released membrane manufacturers from this constraint, leading to the development of membranes known as high-flux membranes that allowed removal of so-called middle molecules, a categorization that has expanded over the years now to include low molecular weight proteins such as β_2-microglobulin (Table 1). Subsequent advances in membrane technology have allowed better control over the structure and permeability of membranes. One consequence of improved membrane engineering has been the development of a new class of membranes, referred to as protein-leaking membranes or super-flux membranes. These membranes are more permeable than conventional high-flux membranes and allow some passage of larger proteins, including albumin. The rationale for these membranes is the need for increased clearance of low molecular weight proteins and protein-bound solutes. Protein-leaking membranes have been fabricated from a variety of polymers, including polymethylmethacrylate, cellulose triacetate, polysulfone, polyarylethersulfone, and polyethersulfone.

Solute transfer across a membrane depends on the properties of the membrane and the mode in which it is operated—in particular, whether the primary mechanism of solute transfer is

Table 1. Classification and typical performance of hemodialysis membranes with respect to protein permeability[a]

	Water Permeability[b] (ml/h per mmHg/m^2)	β_2-Microglobulin Clearance[c] (ml/min)	Albumin Loss[d] (g)	Sieving Coefficient		Application
				β_2-Microglobulin	Albumin	
Low-flux	<6	<10	0		0	HD
High-flux	20 to 40	20 to 40	<0.5	0.7 to 0.8	<0.001	HD, HDF, HF
Protein-leaking	>40	>80	2–6	0.9 to 1.0	0.01 to 0.03	HD

[a]HD, hemodialysis; HDF, hemodiafiltration; HF, hemofiltration.
[b]*In vitro*.
[c]For conventional hemodialysis with a blood flow rate of 300 to 400 ml/min. Includes removal by diffusion, convection, and adsorption.
[d]For 4 h of conventional hemodialysis.

diffusion or convection. Some solute removal may also take place by adsorption to the membrane, depending on the nature of the solute and the membrane. In conventional hemodialysis, solute transfer occurs by diffusion down a concentration gradient between plasma water and dialysate. Diffusion of a solute through a membrane is proportional to the concentration gradient across the membrane, which is determined by the plasma water concentration for solutes not included in the dialysate, the porosity of the membrane, the sieving coefficient for the solute, and the effective diffusivity of the solute in the membrane, and is inversely proportional to the membrane thickness (20). The diffusion coefficient, in turn, is inversely proportional to the size of the solute according to the Stokes-Einstein equation (20). In practice, diffusive mass transfer also is affected by stagnant boundary layers that form at the interfaces between the membrane and the blood and dialysate (21). The extent of boundary layer formation depends on the design of the dialyzer and the flow rates at which it is operated. These membrane and dialyzer design factors are usually combined into a single parameter, the mass transfer–area coefficient (K_oA) of the dialyzer. Because of its dependence on the diffusion coefficient, K_oA decreases rapidly with increasing molecular weight, as shown in Figure 1A, and its importance for large solute removal is limited.

In hemofiltration, transfer of solute occurs by convection down a pressure gradient across the membrane. Convection of a solute through a membrane is proportional to the plasma water concentration of the solute, the porosity and pore radius of the membrane, and the sieving coefficient for that solute and is inversely proportional to the membrane thickness (20). The sieving coefficient is a measure of the steric hindrance between the membrane pores and the solute and, for a given solute, depends on the pore size distribution of the membrane. As shown by the data in Figure 1B, the sieving coefficient decreases more slowly with increasing molecular weight than does K_oA, meaning that convection is less sensitive to molecular size than is diffusion. Sieving coefficients are also affected by boundary layer formation at the blood–membrane interface. As convection occurs, large proteins accumulate at the membrane surface, a phenomenon known as concentration polarization. In the case of a large solute for which the inherent sieving coefficient of the membrane is low, this increase in concentration at the membrane surface may increase the removal rate and the apparent sieving coefficient of the solute. This ability of concentration polarization to increase protein removal has been demonstrated by Kim (22), who found a more than two-fold increase in α_1-microglobulin removal for a 50% increase in filtration rate.

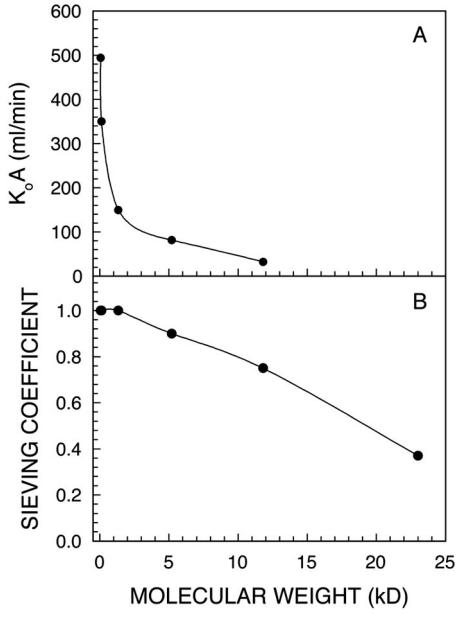

Figure 1. Mass transfer–area coefficient (K_oA; A) and sieving coefficient (B) as function of molecular weight for a typical conventional high-flux hemodialysis membrane. Data points represent average values taken from the manufacturer's literature and published reports.

From this discussion, it is evident that the extent to which a low molecular weight protein is removed by a given membrane will depend on whether the membrane is used in a predominantly diffusive therapy (hemodialysis) or a convective therapy (hemofiltration). In practice, however, solute transfer across a membrane usually occurs by a combination of diffusion and convection. With low-flux membranes, diffusive and convective transfer of low molecular weight proteins is negligible; both K_oA and the sieving coefficient for solutes in this size range are very low because of the large solute size and because the pore size distribution of the membrane is biased toward small diameter pores. With high-flux membranes and protein-leaking membranes, the pore size distribution is shifted toward larger diameter pores, allowing some limited diffusive transfer of low molecular weight proteins. This diffusive transfer is supplemented by uncontrolled convection arising from a process of internal filtration and backfiltration (23). Internal filtration and backfiltration occur when membranes of high water permeability are used with a volume-control system because the low mean transmembrane pressure required to remove the water needed to achieve a patient's dry weight and the countercurrent nature of blood and dialysate flows in a dialyzer create local pressure gradients that favor filtration from blood to dialysate at the blood inlet end of the dialyzer and filtration from dialysate to blood at the blood outlet end of the dialyzer. Because convective transfer occurs at the dialysate outlet end of the dialyzer, convected solutes are swept into the dialysate outflow and effectively cleared. This phenomenon may produce internal filtration rates of up to 30 ml/min (23). Internal filtration is ubiquitous with high-flux membranes and increases in magnitude as the hydraulic permeability of the membrane increases; it may be the primary mechanism of large solute removal in high-flux hemodialysis. Some manufacturers have engineered their membranes and dialyzers to accentuate internal filtration. Reducing the internal diameter of the membrane increases the pressure at the inlet to the blood compartment, leading to an increase in the filtration rate in the proximal section of the hollow fiber and a corresponding increase in the reverse direction at the distal end of the hollow fiber (24).

In hemodiafiltration, diffusion and convection are combined deliberately by superimposing a controlled amount of convection on top of diffusion. An ultrafiltration rate in excess of that required to achieve dry weight is used, with the patient's volume being maintained by infusion of a sterile, pyrogen-free electrolyte solution either before (predilution) or after (postdilution) the filter (25). Replacement solution may be prepackaged or prepared on-line by sequential ultrafiltration of dialysate. In postdilution hemodiafiltration, ultrafiltration rates typically range from 80 to 120 ml/min. Thus, hemodialysis with conventional high-flux membranes and protein-leaking membranes is akin to hemodiafiltration, albeit with an uncontrolled and variable filtration rate.

Albumin loss during hemodialysis with conventional high-flux membranes is generally reported to be in the range of 0 to 2 g per 4-h treatment, depending on the membrane material and the surface area (12,18,26). These findings are consistent with an extremely small K_oA for albumin and limited ultrafiltration. In hemofiltration or hemodiafiltration, however, there may be significantly greater albumin loss with the same membranes because of the higher ultrafiltration rate. Ahrenholz *et al.* (27) measured albumin removal during postdilution hemodiafiltration as a function of filtration rate with a variety of membranes. As the ultrafiltration rate increases from values in the range needed to achieve dry weight in hemodialysis to values typical of on-line hemodiafiltration, albumin removal increased markedly from <2 g/4 h at a filtration rate of 30 ml/min to up to 7 g/4 h at a filtration rate of 90 ml/min. Thus, although convective therapies offer superior removal of low molecular weight proteins, such as β_2-microglobulin, they may also render conventional high-flux membranes protein leaking.

On the basis of the assumption that albumin loss is undesirable, strategies have been developed to minimize albumin loss while maximizing the removal of low molecular weight proteins or protein-bound solutes. Alternating short periods of filtration and backfiltration have been used successfully to reduce albumin loss during hemodiafiltration. This process is referred to as "push/pull" hemodiafiltration. The periods of backfiltration reduce concentration polarization of albumin at the blood–membrane interface, thus reducing the concentration of albumin in the filtrate during filtration. Hemodiafiltration using a polyacrylonitrile membrane with a sieving coefficient of 0.009 for albumin and a substitution solution volume of 20 L resulted in loss of 6.3 ± 3.2 g of albumin into the dialysate in a 4-h treatment (28). Albumin loss with the same membrane operated in a push/pull mode for the same treatment time was 2.2 ± 0.3 g. Although the push/pull process reduced albumin loss, it was associated with an increase in β_2-microglobulin removal, presumably because backfiltration destroys the concentration polarization boundary layer that may effectively reduce the sieving coefficient of the membrane for β_2-microglobulin. Emphasizing the importance of filtration in albumin removal, when the same membrane was used for hemodialysis, albumin loss was only 1.1 ± 0.2 g.

In the past, hemofiltration was often performed in the postdilution mode because it required less replacement solution to provide comparable small solute removal to hemodialysis. On-line fluid production has eliminated concerns over replacement solution volume. Santoro *et al.* (29) showed that predilution hemofiltration with a filtrate volume twice that used in postdilution preserves urea removal and reduces albumin loss from 2.9 ± 1.5 to 1.7 ± 0.8 g without compromising β_2-microglobulin removal. Predilution may also improve removal of protein-bound solutes (30), presumably by shifting solute from the bound to the unbound fraction.

From this discussion, it can be seen that albumin loss in extracorporeal renal replacement therapies is not a simple matter of whether a so-called protein-leaking membrane is used. Some level of albumin loss occurs with all but low-flux membranes. The magnitude of this albumin loss depends on the porosity of the membrane, the length of the treatment, the net fluid removal rate, and the degree to which the treatment modality involves convection.

Technical Considerations

There seem to be few technical barriers to performing routine hemodialysis with dialyzers that contain protein-leaking membranes, unlike on-line hemodiafiltration or hemofiltration, which require specialized equipment to prepare and deliver replacement solution. Dialyzers that contain protein-leaking membranes can be used with any dialysis machine that incorporates a system for close control of fluid removal. Both on-line convective therapies and protein-leaking membranes, however, require careful attention to dialysate purity. Ultrapure dialysate (31) must be used to prepare the replacement fluid that is infused into the blood in on-line convective therapies. Because the large pore size of protein-leaking membranes may facilitate transfer of bacterial products from dialysate to blood, ultrapure dialysate should also be used with protein-leaking membranes. Among other effects, bacterial products from the dialysate may stimulate an inflammatory response and compromise a patient's ability to increase albumin synthesis in response to albumin loss across the protein-leaking membrane (31). Some membrane materials provide a final barrier against endotoxin transfer into the blood by virtue of their capacity to adsorb endotoxin and endotoxin fragments (32). Finally, reuse of dialyzers that contain protein-leaking membranes should be undertaken with great care, if at all, given the documented ability of some reprocessing techniques to increase the protein permeability of some membranes (12,13,16).

Clinical Outcome Studies with Protein-Leaking Membranes

To date, relatively few clinical studies have been designed specifically to test hypotheses that use of protein-leaking membranes affects some clinical outcome in chronic hemodialysis. In addition, there has been little effort to document the level of albumin removal in long-term studies with hemodiafiltration and to correlate that removal with clinical outcomes. Studies that have been conducted with protein-leaking membranes (Table 2) have used changes in some biochemical marker, rather than a clinical outcome, as the primary end point. Seven studies have been reported in full manuscript form (33–40).

Table 2. Clinical studies of protein-leaking membranes[a]

Author (Reference)	End Points	Study Design	No. of Patients	Period of Observation	Comparative Therapy
Niwa *et al.* (33)	CMPF, Hb, Hct	Observational	8	4 mo	Low-flux HD
Locatelli *et al.* (34)	Hb	Randomized	84	3 mo	Low-flux HD
Stein *et al.* (35)	Pentosidine, AGE–β_2-microglobulin	Observational	20	6 mo	High-flux HD
Van Tellingen *et al.* (36)	Hcy	Randomized	30	3 mo	High-flux HD
De Vriese *et al.* (37)	Hcy	Randomized	45	1 mo	Low-flux or high-flux HD
Galli *et al.* (38,40)	Hcy, pentosidine, protein carbonyls, AOPP, cytokines	Randomized	26	6 mo	Low-flux and high-flux HD
Tessitore *et al.* (39)	Pentosidine	Observational	18	7 mo	Low-flux HD

[a]CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; Hb, hemoglobin; Hct, hematocrit; AGE, advanced glycation end products; Hcy, homocysteine; AOPP, advanced oxidation protein products.

Anemia Correction

ESRD is characterized by anemia that arises mainly from an erythropoietin deficiency. The response to erythropoietin replacement is variable, however, and hemoglobin concentrations remain low in some patients despite increasing doses of erythropoietin and adequate iron stores. Uremic toxins with an effective molecular weight in the 30- to 70-kD range may contribute to erythropoietin resistance (33,41). 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) is >98% bound to albumin and has been reported to inhibit erythropoiesis (33). In an observational study, Niwa *et al.* (33) examined the effects of high-flux hemodialysis with a protein-leaking polymethylmethacrylate membrane (BK-F; Toray, Tokyo, Japan) on serum levels of CMPF in eight patients who were treated previously with low-flux cellulose membranes and had hematocrits <27%. Serum levels of CMPF were 10-fold greater than normal at entry to the study and decreased by 51% over 4 mo of treatment with the protein-leaking membrane; 2.8 ± 1.0 mg of CMPF was recovered in the dialysate with the protein-leaking membrane compared with 0.1 ± 0.1 mg with the low-flux membrane. Hemoglobin concentrations increased significantly during the same period, from approximately 8.1 to 8.8 g/dl. In a small number of determinations, 3.5 ± 0.6 g of total protein and 2.5 ± 0.4 g of albumin were lost into the dialysate per treatment with use of the protein-leaking membrane, and this loss was associated with a small but significant decrease in serum albumin and serum total protein concentrations (33). Galli *et al.* (38) obtained similar results in a study in which 26 patients were randomized to treatment with protein-leaking (BK-F) or conventional low-flux and high-flux membranes. The mean hematocrit increased significantly, from 32.5 ± 4.1 to 37.1 ± 3.8%, after 6 mo of hemodialysis with the BK-F membrane. No significant change in hematocrit occurred in the control group, although an explicit statistical comparison between the two groups was not reported.

Subsequent to these small studies, Locatelli *et al*. (34) conducted a multicenter, randomized study to test the effect of using the protein-leaking BK-F membrane on anemia correction. Patients who were being treated with low-flux dialyzers; had a stable hematocrit <30% during the preceding 3 mo; and had no changes in erythropoietin dose, iron, folic acid, or vitamin B_{12} during the same period were randomized to treatment with dialyzers that contained low-flux cellulose membranes or the BK-F membrane. Eighty-four patients were randomized and followed for 12 wk. The primary outcome variable was hemoglobin concentration. The study was powered to detect a difference in hemoglobin concentration of 0.83 g/dl between the two groups. Although hemoglobin concentration increased slightly during the course of the study, neither hemoglobin concentration nor erythropoietin dose differed between the two groups. The total protein concentration decreased during the 12 wk of study in the group that was treated with the protein-leaking membrane, and this change was significant compared with the group that was treated with the low-flux membrane. The albumin concentration also decreased in the group that was treated with the protein-leaking membrane, but this change was NS compared with the group that was treated with the low-flux membrane.

Hyperhomocysteinemia

There is a high prevalence of hyperhomocysteinemia in ESRD patients, and it has been suggested as a risk factor for cardiovascular disease in these patients. A second general hypothesis addressed in clinical trials of protein-leaking membranes is that their use will reduce plasma concentrations of homocysteine, which is 70 to 80% bound to serum proteins. Van Tellingen *et al.* (36) compared changes in plasma total homocysteine after 12 wk of hemodialysis in 30 patients who were randomized to treatment with a conventional high-flux polysulfone membrane or protein-leaking membranes of polysulfone (F 500S; Fresenius, Bad Homburg, Germany) or cellulose triacetate (Tricea 150G; Baxter, Osaka, Japan). There was no difference in total homocysteine among the three groups at entry to the study. After 12 wk, total homocysteine decreased by 33% in the two groups that were treated with the protein-leaking membranes but remained unchanged in the group that was treated with the conventional high-flux polysulfone membrane. The difference between the conventional high-flux membrane and the protein-leaking membrane was highly significant and remained so after adjustment for vitamin co-factors, serum

albumin, age, gender, dialysis vintage, and urea Kt/V. Subsequently, Galli *et al.* (38) performed a similar study in which 26 patients were randomly assigned to treatment for 6 mo by hemodialysis with conventional membranes or the protein-leaking BK-F membrane. These investigators also found a decrease in total homocysteine concentration (32% at 6 mo) with the protein-leaking membrane and no change with the conventional membranes. Again, the difference between the two membranes was significant. The median amount of protein removed during a treatment with the protein-leaking membrane was 2.86 g compared with 0.26 g with the conventional membranes. This protein loss was associated with an initial decrease in serum albumin concentration; however, serum albumin had stabilized at a level 5% lower than the level at entry by the end of the 6-mo study period and was not different from that in the control group. In another study, De Vriese *et al.* (37) assigned 45 patients to 4-wk blocks of dialysis with dialyzers that contained low-flux, high-flux, and protein-leaking membranes of cellulose triacetate (Sureflux-150FH; Nipro, Osaka, Japan). The protein-leaking membrane was associated with a $15 \pm 3\%$ decrease in pretreatment plasma total homocysteine concentrations, compared with no change with the low-flux and high-flux membranes ($P < 0.001$). There was no detectable loss of albumin into the dialysate with the low-flux and high-flux membranes, whereas 5.2 ± 0.4 g per treatment was recovered in the dialysate with the protein-leaking membrane. This loss of albumin did not affect the serum albumin concentration during the 4 wk of dialysis with the protein-leaking membrane.

Protein Oxidation and Glycation

ESRD is associated with chronic inflammation, which has been suggested as another risk factor for cardiovascular disease in ESRD patients. Oxidized and glycosylated proteins are thought to be mediators of inflammation in ESRD. Three studies have examined the impact of protein-leaking membranes on plasma concentrations of advanced glycation end products (AGE). Ten nondiabetic hemodialysis patients who were being treated with low-flux membranes were switched to high-flux membranes (Superflux 800S; Fresenius) for 6 mo, followed by protein-leaking membranes for an additional 6 mo (35). A second group of 10 diabetic patients were switched from low-flux membranes to the protein-leaking membrane for 6 mo, followed by high-flux membranes for 6 mo. Plasma concentrations of free and protein-bound pentosidine and AGE–β_2-microglobulin were used to assess AGE levels. Use of the protein-leaking membrane was associated with a modest decrease in the concentrations of free and protein-bound pentosidine compared with use of the low-flux membrane. Use of the conventional high-flux membrane did not affect pentosidine levels. The protein-leaking membrane had minimal impact on plasma concentrations of AGE–β_2-microglobulin. More recently, Tessitore *et al.* (39) reported changes in free and protein-bound pentosidine in 18 patients in a crossover study in which hemodialysis patients were treated alternately with low-flux or protein-leaking membranes (BK-F) for periods of 7 mo. In this study, use of the protein-leaking membrane was also associated with a reduction in the predialysis concentrations of free (24%) and protein-bound (18%) pentosidine compared with when a low-flux membrane was used. Finally, Galli *et al.* (40) determined plasma concentrations of pentosidine, protein carbonyls, and advanced oxidation protein products in the same patients in whom they examined changes in homocysteine levels (see previous section). Plasma concentrations of free and albumin-bound pentosidine, protein carbonyls, and advanced oxidation protein products, which were significantly greater than normal at entry to the study, decreased significantly over 6 mo in the 13 patients who were treated with the protein-leaking membrane, while remaining unchanged in the 13 patients who were treated with conventional membranes. Plasma concentrations of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) also decreased in the protein-leaking group, whereas the concentration of IL-10 increased (40).

Summary

Taken together, these reports support the hypothesis that protein-leaking membranes are of benefit in reducing concentrations of solutes that are thought to contribute to morbidity in chronic hemodialysis patients. However, the findings are not conclusive. The study of Locatelli *et al.* (34), which is the only randomized, multicenter study reported to date, did not show a benefit of using protein-leaking membranes. The remaining studies are single center, and several are only observational in nature. Furthermore, the number of patients studied is small, and the longest follow-up is only 7 mo (Table 2). All studies that have been published so far rely on laboratory measures as the primary outcome. None has used a clinical event, such as mortality or nonfatal cardiovascular event, as the outcome variable. It is not known whether use of protein-leaking membranes for longer times will continue to lower solute concentrations or whether the decreases in solute concentrations achieved with protein-leaking membranes will translate into improved clinical outcomes. In short, important questions still need to be answered before the chronic use of protein-leaking membranes could be considered to have demonstrated clinical benefit. Some of these questions are addressed in the following paragraphs.

Unresolved Questions

What Are the Targets for Protein-Leaking Membranes?

The rationale for using protein-leaking membranes is that they allow increased removal of important uremic toxins. Just what those toxins might be and how they manifest their toxicity are not entirely clear. The suggestion is that they are small protein-bound solutes or solutes in the 35- to 60-kD size range. Whereas a number of candidates for the former group have been identified (1), few candidate toxins with molecular weights >35 kD have emerged. Indeed, the list of uremic toxins compiled by the European Uremic Toxin Work Group contains no solute in the 35- to 60-kD size range (1). Alternatively, protein-leaking membranes might allow increased removal of lower molecular weight proteins, such as β_2-microglobulin. Changing from a conventional high-flux membrane to the BK-F protein-leaking membrane for hemodialysis doubled the pre- to postdialysis decrease in plasma

β_2-microglobulin concentration during a 4-h treatment at the cost of losing 3.3 g of albumin into the dialysate (42). However, changing from a high-flux to a protein-leaking membrane for hemodiafiltration resulted in a very modest increase in β_2-microglobulin removal, whereas albumin loss increased from 2 to 8 g/4 h treatment (27). These results are not surprising because high-flux membranes already have a high sieving coefficient for β_2-microglobulin, whereas the K_oA for β_2-microglobulin is much lower than that provided by protein-leaking membranes.

It might be argued that the improved responsiveness to erythropoietin reported with use of protein-leaking membranes demonstrates that there are important uremic toxins with an effective size of 35 to 60 kD. Galli *et al.* (41) isolated polyamine-protein conjugates that inhibit erythroid colony-forming units; however, the inhibitory activity seems to be confined to substances of >200 kD, which would not be removed with current protein-leaking membranes. Moreover, there is an alternative explanation for the improved response to erythropoietin observed with protein-leaking membranes. Use of ultrapure dialysate has been shown to improve responsiveness to erythropoietin, possibly by reducing inflammation (43). If use of protein-leaking membranes were also accompanied by a change to ultrapure dialysate, then the ultrapure dialysate rather than the protein-leaking membrane might explain the improved responsiveness to erythropoietin. No information on dialysate quality is available for the studies reported so far (33,34,38).

Homocysteine provides some support for the presence of high molecular weight uremic toxins. Homocysteine is a small solute that is 70 to 80% protein bound. It has been hypothesized that the pre- to postdialysis reduction in plasma total homocysteine concentration observed with conventional high-flux membranes results from removal of large molecular weight inhibitors of enzymes involved in the metabolic clearance of homocysteine, rather than direct dialytic removal of free homocysteine (44). The reduction in predialysis plasma total homocysteine concentration reported with protein-leaking membranes (36–38) is consistent with this hypothesis. However, these putative inhibitors remain to be characterized, and their molecular weight is uncertain.

More work is needed to identify solutes with demonstrated toxicity in the size range cleared by protein-leaking membranes. Understanding whether it is desirable to remove solutes in the 35- to 60-kD size range as distinct from small solutes that acquire an effectively large size because of protein binding is an important goal, because there may be alternative strategies for removing protein-bound solutes that do not involve protein-leaking membranes. For example, removal of protein-bound solutes may be increased using predilution hemofiltration or hemodiafiltration with a conventional high-flux membrane (30,45). One potential area for further work involves proteins in the 30- to 60-kD size range that are present in uremic plasma in essentially normal concentrations but in posttranslationally modified forms (5,6).

Can Protein-Leaking Membranes Have an Impact on Uremic Toxins the Size of Albumin?

Albumin may play a role in uremic toxicity, because it serves either as a carrier protein for small, highly protein-bound uremic toxins (8,46) or directly as a result of posttranslational modification (5,6,47). Low molecular weight albumin-bound uremic toxins may be removed by protein-leaking membranes by virtue of the internal filtration and backfiltration that occur with highly permeable membranes. Replacement of plasma water with filtered dialysate results in a shift of solute from the albumin-bound to the unbound state and subsequent removal by diffusion and convection. This phenomenon has been demonstrated for p-cresol, which is >75% protein bound, using predilution hemodiafiltration (30). The ability of internal filtration to increase albumin-bound solute removal depends on the binding kinetics of the solute. For example, homocysteine is not easily displaced from albumin (48), and the amount of homocysteine recovered in dialysate is not markedly different among low-flux, high-flux, and protein-leaking membranes (37), suggesting that internal filtration and backfiltration are not responsible for the decrease in plasma total homocysteine concentration observed with protein-leaking membranes (36–38).

Some investigators have reported that use of protein-leaking membranes reduces plasma concentrations of biochemically modified proteins (35,39,40). Albumin comprises the majority of these modified proteins because of its high abundance in plasma relative to other proteins. For example, plasma pentosidine exists 90% in the albumin fraction (47), and albumin is the major plasma protein target for oxidation (5). The amount of albumin removed by protein-leaking membranes (2 to 5 g/treatment) is too small to account for the decrease in modified albumin merely by increasing albumin turnover. Furthermore, protein-leaking membranes cannot selectively reduce the plasma concentration of modified albumin relative to native albumin. Because albumin modified by oxidation or glycation does not differ markedly in molecular size from native albumin (5,49), modified albumin and native albumin will be removed in the same ratio as their plasma concentrations. These observations suggest that protein-leaking membranes have an effect on modified albumin that is more complex than simple removal of albumin across the dialyzer membrane. One possible explanation for the decrease in modified albumin observed with protein-leaking membranes is that these membranes remove proinflammatory and pro-oxidant mediators, thereby reducing inflammation and oxidant stress and albumin glycation and oxidation. In support of this hypothesis, Galli *et al.* (40) reported a decrease in plasma concentrations of proinflammatory cytokines in patients who were treated with protein-leaking membranes, apparently unrelated to dialytic removal. Further studies are required to identify these immunomodulatory and pro-oxidant solutes and to demonstrate that their removal does indeed decrease the biochemical modification of albumin. An alternative hypothesis is that the use of ultrapure dialysate with protein-leaking membranes reduces inflammation and oxidant stress (31). This mechanism seems unlikely, however, because use of protein-leaking membranes is associated with lower levels of modified albumin than conventional hemodialysis

when dialysate of equal microbiologic quality is used for both therapies (39,40).

Protein-Leaking Membranes, Convective Therapies, or Both? Even if long-term treatment with protein-leaking membranes is sufficient to improve clinical outcomes by reducing concentrations of higher molecular weight uremic toxins, it is not clear whether using this new class of membranes is necessary. Most studies in Table 2 compare hemodialysis with protein-leaking membranes with hemodialysis using low-flux membranes. The wide disparity in high molecular weight clearances between the two classes of membrane leaves unanswered questions about how much high molecular weight solute removal is needed and the size range of the solutes that must be removed to provide the effect reported for protein-leaking membranes. It is unclear whether there is diffusive loss of high molecular weight solutes with protein-leaking membranes or there is just more internal filtration as a result of the higher water permeability of the membrane. In the latter case, conventional high-flux membranes might provide the same benefit if internal filtration were maximized or if they were used for convective therapies, such as hemofiltration and hemodiafiltration.

Some evidence that this new class of protein-leaking membranes may not be necessary for clinical improvement can be found in the case of anemia correction. An improved response to erythropoietin has been reported in two observational studies of patients who were treated by on-line hemodiafiltration with conventional high-flux membranes (50,51), although the finding was not confirmed in a randomized, controlled study (52), and there is the potentially confounding effect of ultrapure dialysate to consider (31). A preliminary report also suggested that plasma total homocysteine concentrations may be normalized with a combination of on-line hemodiafiltration and vitamin supplementation (53). Finally and beyond the scope of this review is the alternative strategy of increasing the removal of low molecular weight proteins and improving outcomes by increasing the frequency of treatment from thrice weekly to 6 d/wk (54).

How Much Albumin Loss Is Acceptable? The maximum level of albumin loss that should be tolerated with albumin-losing therapies has yet to be established (55). Albumin loss during on-line hemodiafiltration with conventional high-flux membranes ranges from 1 to 3 g per treatment (56). With this level of albumin loss, serum albumin concentrations are stable in patients who are treated long term with on-line hemodiafiltration (57). The situation with protein-leaking membranes is less clear. In five of the seven studies listed in Table 2, no significant change was found in serum albumin concentration with albumin losses in the range of 2 to 5 g/treatment (34–38). However, the duration of these studies is short, and there was a trend for serum albumin concentrations to decrease in two of them (34,38). In the remaining two studies (33,39), which were small, serum albumin concentration did decrease significantly, by approximately 10%, with likely similar albumin losses. The patients who participated in all seven studies had serum albumin concentrations in the normal range at entry to the study, and a low serum albumin was an exclusion criterion in two studies (36,38,40).

So far, the preponderance of data are that use of protein-leaking membranes for conventional hemodialysis tends to result in a small decrease in serum albumin concentration of, so far, unknown consequence, at least in well-nourished patients. Could protein-leaking membranes be used for hemodiafiltration? Use of protein-leaking membranes for hemodiafiltration results in an albumin loss of up to 7 g/treatment (28,56,58). To date, there are no long-term studies to show whether this level of albumin loss has an impact on serum albumin. The level of protein loss is still well below the 10- to 12-g/treatment reported to occur after dialyzers that contain a polysulfone membrane are cleaned multiple times with bleach (12,13,16), and which was associated with a 0.22- to 0.25-g/dl decrease in serum albumin concentration (12,13). However, the average albumin loss experienced by these patients may have been much less than 10 to 12 g/treatment because it took 20 to 25 uses of the dialyzer to achieve that level of loss.

These data provide some general guidance on the relationship between albumin loss and serum albumin; however, more study is required to establish clearly the upper limit for acceptable albumin loss and to define those groups of patients who should not be treated with protein-leaking membranes because of an inability to increase appropriately albumin synthesis. For example, it may be prudent to limit the use of albumin-losing therapies in malnourished patients. Finally, there is no information so far on potential adverse effects of removing other proteins of similar size to albumin.

Conclusions

A new class of membranes that leak protein is becoming available for chronic hemodialysis. These membranes provide greater clearances of low molecular weight proteins and protein-bound solutes but at the price of some albumin loss. Protein-leaking membranes have been used in a small number of clinical trials that have focused mostly on anemia correction and plasma concentrations of homocysteine and oxidized and glycosylated proteins. The data reported so far are provocative but do not make a compelling case for chronic use of these membranes. Uremic toxins that are not removed by conventional high-flux membranes and that might be removed by protein-leaking membranes have yet to be identified. Also, it is unclear whether protein-leaking membranes offer benefits beyond those that might be obtained using conventional high-flux membranes in therapies that are designed to increase convective solute removal. Finally, how much albumin loss should be tolerated in ESRD patients and whether the magnitude of this loss varies from patient to patient remain to be determined. Further research to address these questions is needed.

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