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Clinical Significance of a Dialysis Membrane That Can Remove β_2-Microglobulin (β_2m)

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Through clinical and *in vitro* evaluations clinical significance of continued use of a β_2m removal membrane was validated. *ASAIO Transactions* 1988; 34: 342–345.

Identification of β_2-microglobulin (β_2m) as a major component of amyloid fibrils in a chronic dialysis patient with carpal tunnel syndrome[1] demonstrated the importance of identifying a pathogenic substance, *i.e.*, a series of observations that support or suggest the pathogenicity of β_2m quickly followed,[2-5] although it is not wholly proven yet. On the other hand, with encouragement by these publications, various clinical attempts to remove β_2m from chronic dialysis patients have been undertaken.

However, none of these trials, including continued use of a membrane able to remove β_2m, have succeeded in correcting the high plasma levels of β_2m to normal, because of the "leveling-off phenomenon" before reaching this goal. We therefore intended to provide insight into the clinical significance of such a β_2m-removal membrane through clinical and *in vitro* evaluation.

Methods

Materials

As a β_2m-removal dialysis membrane, a poly(methyl methacrylate) (PMMA) membrane, "Filtryzer" BK (Toray Industries, Inc.), with a surface area of 1.0 ~ 2.0 m^2 was used, as well as a conventional cellulosic membrane serving as a control. According to differential scanning calorimetric analysis (DSC), the pore radius of the BK membrane was found to be 40 ~ 70 Å.

All clinical data were collected under conventional hemodialysis conditions at blood and dialysate flow rates of 200 and 500 ml/min, respectively.

Analysis

The concentration of β_2m in plasma and dialysate was measured by radioimmunoassay; the dialysate samples (60 ml) were lyophilyzed, resolubilized into distilled water (3 ml), and then measured, whereas such pretreatment was not applied to the plasma.

Because hemoconcentration occurs during dialysis, the percent reduction of β_2m in plasma was defined as follows:

$$\left(1 - \frac{[\beta_2\text{m}]_{post}}{[\beta_2\text{m}]_{pre}} \times \frac{[\text{TP}]_{pre}}{[\text{TP}]_{post}}\right) \times 100$$

where TP, [ ] pre, and [ ] post mean total protein in plasma, plasma concentration at predialysis and postdialysis, respectively.

Subjects

In long-term clinical evaluation, three men in each group (A and B) were studied, where the duration on dialysis was >4 (4 ~ 12) and <1 year for Groups A and B, respectively. In a short-term study, patients on dialysis who were just beginning on dialysis were examined.

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

Effect of Continued Use of a β_2m-Removal Membrane on Plasma β_2m Levels

When patients in Groups A and B were switched from treatment with a cellulosic membrane (1.5 m^2) to BK-1.0H (1.0 m^2), plasma β_2m levels decreased in all without any difference in the pattern seen between long and short durations of dialysis treatment, as shown in **Figure 1.** For the three patients in Group B, BK-1.0H was replaced with a cellulosic membrane after use of the BK-1.0H for 5 months. **Figure 1** also shows that plasma β_2m in Group B gradually increased, whereas that in Group A, maintained on a BK-1.0H membrane, remained unchanged. It is also observed that, despite continued use of the β_2m-removal membrane, plasma β_2m leveled off before reaching the normal range. Therefore, in order to evaluate removability of β_2m by a dialysis membrane, BK-1.5 (1.5 m^2) was prescribed

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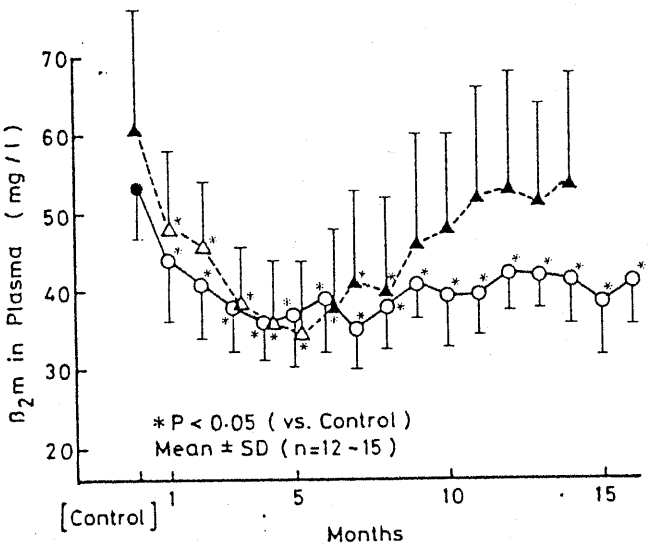

Figure 1. Changes in plasma β_2m in two groups of patients on dialysis: Groups A (duration: more than 4 years) and B (less than 1 year). *Circles* and *triangles* designate data for Groups A and B, respectively, and *open* and *closed* for β_2m-removal membrane (BK) and control cellulosic membrane, respectively.

for three patients in Group A after 17 months use of a BK-1.0H. Changes in plasma levels of β_2m, as well as its reduction during the two phases of the study, are listed in **Table 1.** There was a significantly enhanced reduction rate when using the BK-1.5 as compared with the BK-1.0H, and a slight but significant decrease in plasma β_2m was observed. However, the leveling off phenomenon was again observed.

Changes in Plasma β_2m During Dialysis Treatment

To clarify this leveling off phenomenon, *in vitro* experiments corresponding to those carried out in the clinical setting were performed. Pooled plasma (2.8 L) collected from patients on dialysis was dialyzed with a BK-1.0H at plasma and dialysate flow rates of 150 and 500 ml/min, respectively, and at ultrafiltration rates of 0 and 40 ml/min. Clinical data obtained with the BK-1.0H and BK-2.0 (2.0 m^2) were compared with those of the above *in vitro* experiment. A remarkable contrast in decreasing patterns between clinical and *in vitro* settings is shown in **Figure 2.** This comparative evaluation suggests an increased flow of β_2m into the blood compartment and/or its enhanced generation in the clinical setting.

Table 1. Changes in the Reduction Rate of β_2m During Dialysis and its Predialysis Plasma Level by Replacing a Conventional Cellulosic Membrane (1.5 m^2) with β_2m-Removable Membrane, BK-1.0H (1.0 m^2) and then BK-1.5 (1.5 m^2)

	β_2m Reduction Rate (%)	β_2m in Plasma (mg/L)
Control	—	54 ± 7 (12)
BK-1.0H		
Month 1	41.8 ± 8.1 (9)	44 ± 9* (9)
Month 2	37.0 ± 7.2 (12)	41 ± 7* (12)
Month 3	38.3 ± 7.6 (15)	38 ± 6* (15)
Month 17	36.6 ± 5.2 (33)	41 ± 4* (21)
BK-1.5		
Month 1	52.6 ± 5.2† (12)	38 ± 4† (12)
Month 2	50.3 ± 4.2† (12)	36 ± 4† (12)
Month 3	50.5 ± 4.3† (12)	37 ± 4† (12)
Month 4	49.3 ± 3.8† (12)	36 ± 4† (12)

* $P < 0.05$ (*vs.* control).
† $P < 0.05$ (*vs.* BK-1.0H month 17).

Rebound of Plasma β_2m After the End of Dialysis

As the next step, intradialytic and interdialytic plasma levels of β_2m, blood urea nitrigen (BUN), and total protein (TP) were measured in four patients on dialysis who had been treated with, first, a cellulosic membrane (1.0 m^2) and then with a BK-2.0 for the first time. As shown in **Figure 3,** in contrast to BUN, which linearly increased after the end of dialysis, plasma β_2m rebounded soon after the end of treatment and then remained almost unchanged until the next dialysis. Incomplete recovery of plasma levels of β_2m and BUN to predialysis levels may be explained by the fact that those patients were switched from a dialyzer with a surface area of 1.0 m^2 to that of 2.0 m^2.

Effect of Timing of Introduction of the β_2m-Removal Membrane on Plasma Levels

Based upon our hypothesis that the earlier a β_2m-removal membrane is introduced, the lower the plasma level of β_2m reached will be, two groups of patients were studied. Duration of dialysis with a cellulosic membrane (1.5 m^2) before introduction of BK-1.6P (1.6 m^2) was 6.6 ± 2.2 months for five patients and 20.5 ± 7.0 for another four patients. Although data are still limited, the plasma β_2m level reached by the former group seems to be lower than that of the latter, as shown in **Figure 4.**

Discussion

As reviewed by Ritz and Bommer,[6] dynamics of β_2m accumulation in patients on dialysis and the mechanisms of local deposition of β_2m derived amyloid are not yet clarified, despite many clinical and basic science evaluations encouraged by the identification of this substance.[1] In this study, we tried to find a new clue to the dynamics of β_2m accumulation, as well as establish a practical and effective way to remove β_2m from patients on dialysis.

We observed the following three dynamic phenomena: 1) the decrease in plasma β_2m during clinical dialysis using a β_2m-removal membrane is far less than that in corresponding *in vitro* experiments or than expected from its actual loss into dialysate and estimated adsorption by the membrane. In 36 dialysis treatments using a 1.0 m^2 BK, a mean of 50.4 ± 20.5 mg of β_2m per treatment was detected in the dialysate. On the other hand, it was observed that about 100 mg of β_2m is adsorbed by this membrane in *in vitro* experiments.

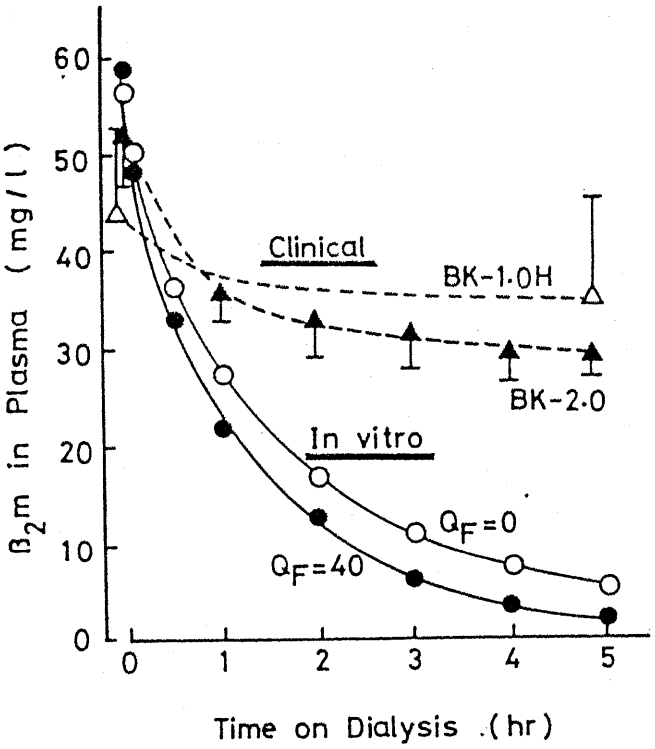

Figure 2. Changes in β_2m concentration in plasma in both clinical and *in vitro* settings. Clinical data were obtained with BK-1.0H in three patients (duration: more than 4 years) and with BK-2.0 in four patients being dialyzed for the first time. *In vitro* dialysis experiments were performed at plasma and dialysate flow rates of 150 and 500 ml/min, respectively, with 2.8 L pooled plasma collected from patients on dialysis.

In the case of the 2.0 m^2 dialyzes, about 400 mg of β_2m is estimated to be removed by both permeation and adsorption. However, the apparent removal of β_2m from the blood compartment, normalized for hemoconcentration by total protein levels, was 48 ± 8 (N = 12) and 67 ± 11 mg (N = 12) during 5 hr dialyses with 1.0 and 2.0 m^2 BK membranes, respectively, which suggests that β_2m of 100 ~ 300 mg enters and/or is generated in the blood compartment during dialysis. 2) Very rapid rebound of β_2m levels soon after the end of dialysis with BK occurs. 3) After the rapid rebound, the increase in plasma β_2m is very slight all through the remaining interdialytic period (about 40 hr). The average increase in plasma β_2m during this period was 110 μg/L/hr, which, if it is considered as a static generation rate, seems to conflict with the daily generation rate already reported[7]; this discrepancy cannot be explained even if β_2m is distributed in total extracellular volume. Therefore, unless the generation of β_2m is intermittent or fluctuates significantly, these three phenomena prompt us to imagine the existence of a "movable" pool of β_2m.

On the other hand, a decrease in predialysis plasma β_2m by continued use of a BK membrane, which was clearly demonstrated in this study, and normalization of plasma β_2m by successful kidney transplantation suggest that such a

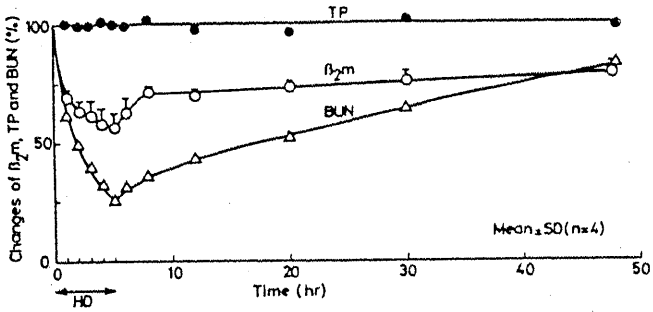

Figure 3. Changes of relative plasma concentrations of total protein (TP), β_2m, and blood urea nitrogen (BUN) during intradialytic and interdialytic phases observed in four patients on dialysis.

movable pool of β_2m, if it exists, is accessible through these measures.

As to the development of a therapeutic method, we carefully observed the continued use of a high flux BK membrane in a conventional dialysis mode. Any adverse effect, such as a fever, which was suggested to result from backfiltration, or a depletion syndrome, has never been observed during a total of 2 yr continued use of BK. To assure ourselves that backfiltration was not a problem, we performed *in vitro* experiments with the BK, where a limulus based endotoxin assay kit, "Pyrodick" (Teikokuzoki Pharmaceutical, Japan), was used for measurement of endotoxin and confirmed that endotoxin that was placed into the dialysate at a concentration of 23 ng/ml was not detected in the blood compartment at a dialysate flow rate of 500 ml/min nor at the reverse ultrafiltration rate of 100 mg/min for 2 hr. In addition to accumulation and/or deposition of intact β_2m in patients on dialysis,[1,2] its fragments have been detected as well.[3,8] Although we have not, as yet, measured the change in plasma levels of those fragments, we consider that bulk removal of intact β_2m may also contribute to lowering the concentration of these alternate forms of β_2m. As it seems to be safe and effective, the continued use of a β_2m-removal membrane, such as the BK, from initiation of dialysis treat-

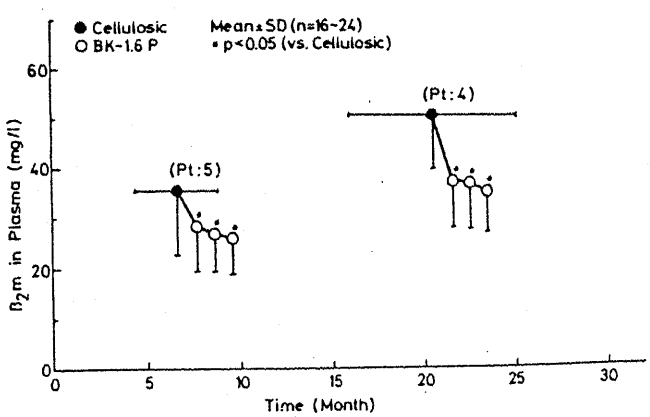

Figure 4. Changes in plasma β_2m when a cellulosic membrane (1.5 m^2) was replaced with a BK-1.6P (1.6 m^2) as a function of time after initiation of dialysis.

ment is recommended as a way to remove β_2m that otherwise accumulates in dialysis patients with time.

Acknowledgments

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Discussion

DR. STEPHEN: I have two questions that tie in to a certain extent. I would like to know some characteristics of those BK membranes, particularly the ultrafiltration index. And, what was the average amount of fluid removed from each patient during treatment? Have you any idea? I realize this question is a little more vague, at least the answer must be. Could you give us some idea on those?

DR. ONO: Dr. Ueno will answer your questions (Kohki Ueno, MD, Toray Industries [America], Inc., New York, New York).

DR. UENO: Mr. Chairman, with your permission, I'd like to help Dr. Ono.

DR. HENDERSON: Certainly.

DR. UENO: The ultrafiltrate rate is about 40 ml/minute.

DR. STEPHEN: That's with 1 mmHg?

DR. UENO: Yes, 1 mmHg.

DR. STEPHEN: Okay, 1 minute?

DR. HENDERSON: I believe we can assume that it is probably a relatively high flux membrane given the PMMA designation.

DR. UENO: Two to three liters of body fluid removal per procedure.

DR. STEPHEN: Thank you very much.

DR. ROECKEL: Can you tell us something about the mode of elimination by the PMMA membrane? We did not observe transmembrane beta-2 M transport during hemodialysis using a PMMA membrane, but we could find high arteriovenous differences in beta-2 M concentration throughout a 4 hour hemodialysis, hinting at a high adsorptive capacity of this low molecular weight function.

DR. UENO: Is your question the way beta-2 is removed?

DR. ROECKEL: By adsorption?

DR. UENO: I'm not sure of your question.

DR. HENDERSON: Did the beta-2 adhere to the membrane or was it transported through the membrane?

DR. UENO: This material has the capability of dropping the material, as well as transporting it through the membrane.

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