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Long-Term Multicentre Study on β_2-Microglobulin Removal by PMMA BK Membrane

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Abstract. A long-term multi-centre clinical study was performed, based upon our first identification of β_2-microglobulin (β_2-M) amyloid fibrils and optimisation of the pore-size of the polymethylmethacrylate (PMMA) membrane for its removal. To clarify the clinical significance of the newly designed PMMA membrane, BK membrane, having an average pore radius of 70–80 Ångströms (7–8 nm), a total of 73 chronic haemodialysis patients from 28 centres, classified into four groups, were studied.

Although the plasma β_2-M decreased in all groups through the continued use of BK membrane, the early introduction of haemodialysis with BK membrane reduced the plasma concentration of this substance more than in the case of the later introduction. Pain index, defined as the total pain score divided by the number of painful joints, decreased significantly over the period of haemodialysis duration with BK membrane. This suggests that the continued use of BK membrane from the early phase of haemodialysis treatment results in the amelioration and/or prevention of joint pains of haemodialysis patients as well as preventing the increase in plasma β_2-M.

Key words: β_2-Microglobulin; Haemodialysis; HD-related amyloidosis; Pain index; PMMA membrane

Introduction

β_2-Microglobulin (β_2-M), which accumulates in chronic haemodialysis patients, was first identified by us in 1985 as a major constituent of amyloid fibrils in haemodialysis-related amyloidosis [1].

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Among the researchers studying the pathophysiology of haemodialysis, actual proof of middle-molecule hypothesis [2] has been one of the ultimate targets for a long time. Our first report on the identification of β_2-M [1] was followed by many pathological, clinical and basic papers on this substance from all over the world. Among them was the detection of this substance from bone cysts and kidney stones by Gorevic et al [3] and Linke et al [4]. On the other hand we attempted to elucidate the effect of β_2-M on bone metabolism in an in vitro setting by using mouse calvaria-derived osteoblastic cells, clone MC3T3-E1 [5]. At a β_2-M concentration of $2–3 \times 10^{-2}$ mg/ml, which corresponds to the plasma β_2-M value found in haemodialysis patients, its inhibitory effect on calcification was detected, while cell proliferation, collagen synthesis, and alkaline phosphatase activity remained unchanged. Furthermore, Floege confirmed the accumulation of β_2-M in joint and bone lesions in haemodialysis patients using ^{131}I-β_2-M injections [6]. These results strongly supported the key role of β_2-M in haemodialysis-related amyloidosis. This remarkably increased interest in β_2-M during the past few years might be the first evidence supporting the middle-molecule hypothesis.

Following our first identification of β_2-M, designing of an optimal membrane to remove this substance has been attempted and polymethylmethacrylate (PMMA) membrane (BK membrane) was developed in 1986. As compared to B1 and B2 membranes [7], which were developed with the same polymer material, PMMA, as a high-flux type and a conventional type, respectively, BK membrane has a bigger pore-size [8] and its characteristic feature is its ability to remove β_2-M through two kinds of mechanisms, that is, adsorption and permeation [9].

To clarify the clinical significance of β_2-M removal by such a leaky membrane, a long-term clinical study using BK membrane was started in 1988. This paper describes

the results of the long-term multicentre study on β_2-M removal by BK membrane.

Methods and Subjects

BK membrane used in this study is made with PMMA, as are B1 and B2 membranes [7]. However, the pore radius of BK membrane, which was determined by differential scanning calorimetric analysis (DSC), is about 70–80 Å(7–8nm), as shown in Fig. 1. [8]. As PMMA was demonstrated to adsorb β_2-M significantly [10], this pore-size has been optimised by taking into consideration its balancing capacity to permeate and to adsorb β_2-M. Scanning electron-micrographs of B2 and BK membranes with a magnification scale are shown in Fig. 2. Differences between the two membranes is clearly seen. However, as reported in our other paper in this issue [11] big pore-like structures seen on the surface of BK membrane do not penetrate through the whole membrane.

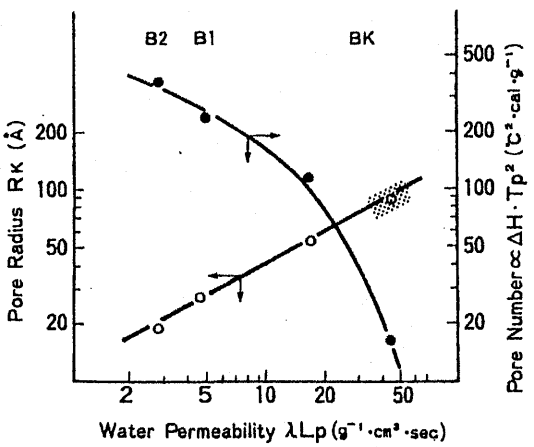

Fig. 1. Relationship between water permeability of B1, B2 and BK membranes with their pore radius (open circles) and pore number (closed circles) respectively.

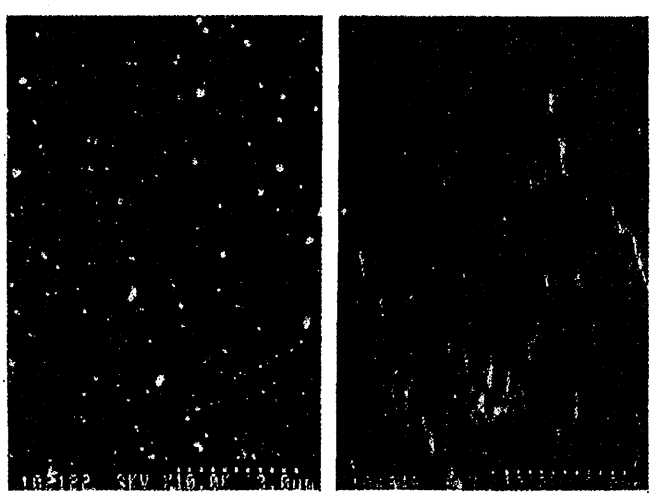

Fig. 2. Scanning electron micrographs of the surface of B2 and BK membranes. Magnification scales are shown in the photographs.

The outline of protocol for our long-term multicentre clinical study of BK membrane is shown in Table 1. Groups A and D were newly introduced haemodialysis patients with BK and conventional membranes respectively. The patients with various joint pains, who had been under haemodialysis treatment for more than 5 years, were classified as group B. The patients dialysed for more than 1 year and without joint pains were included in group C.

Table 1. Summary of HD patient groups using BK and conventional membranes

Group	HD duration	Patients (n)	Age (years)	Joint pain
A	<2 months	10	54±13	—
B	>5 years	43	55±11	+
C	>1 year	11	56±13	—
D	0	9	49±15	—

A,B,C: HD with BK membrane
D : HD with conventional membrane

In this clinical study, plasma β_2-M concentration and scoring of joint pains were the major items for evaluation. For the latter, the regions including neck, shoulder, elbow, wrist, finger, back, hip, coxa, knee, ankle, and toe were observed and pains were scored on five grades by patients' complaints and doctors' evaluation:

Score grade	5: Pain	(+++)	intolerable
	4:	(++)	tolerable
	3:	(+)	slight
	2:	(±)	occasionally slight
	1:	(−)	none

Pain index, S/J, was defined as the total pain score (total of 5 grade score) (S) divided by the number of painful joints (J). Decrease of S/J by more than 2 was interpreted as improved.

All data were expressed as mean ± standard deviation (SD) and statistical difference was studied by Student's t-test and χ^2 test.

Results

Plasma β_2-M values in groups A and D are plotted as a function of haemodialysis duration in Fig. 3. Plasma β_2-M values remained unchanged in group A patients, who were introduced to haemodialysis treatment with BK membrane (19.8 ± 9.1 ml/l at the time of haemodialysis

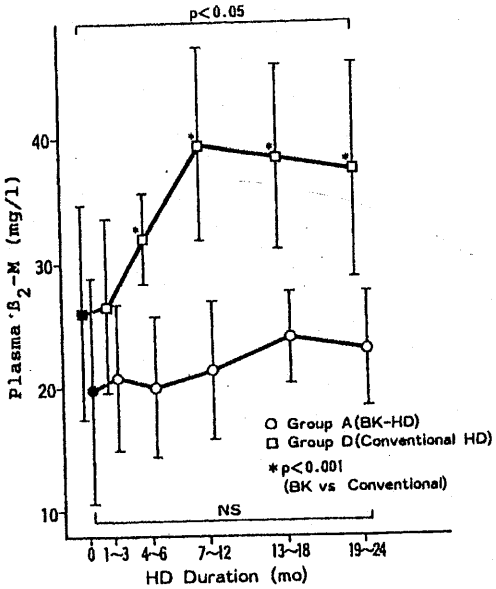

Fig. 3. Changes in plasma β_2-M levels in patients of groups A and D started on haemodialysis with BK and conventional membrane respectively.

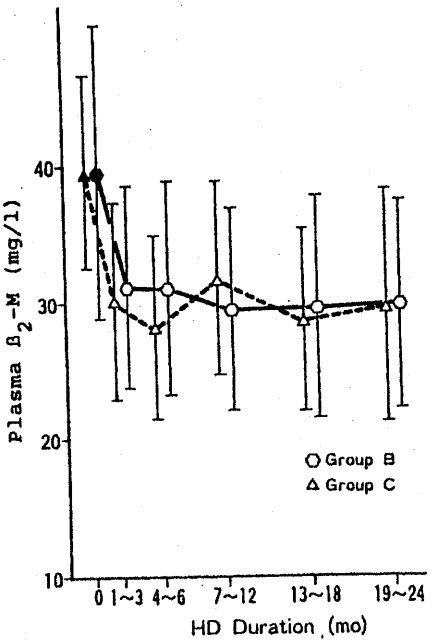

Fig. 4. Changes in plasma β.M levels in patients of groups B and C when they were changed from haemodialysis with conventional membrane to haemodialysis with BK membrane.

introduction, 23.1 ± 4.6 mg/l after 19–24 months). On the other hand, values increased significantly in group D patients treated with conventional membrane haemodialysis, when haemodialysis duration exceeded 4–6 months (26.1 ± 8.6 mg/l at the time of haemodialysis introduction, 32.0 ± 3.6 mg/l after 4–6 months). Maximal values were reached after 7–12 months from the onset of haemodialysis treatment (39.5 ± 7.7 mg/l), and maintained during the observation period.

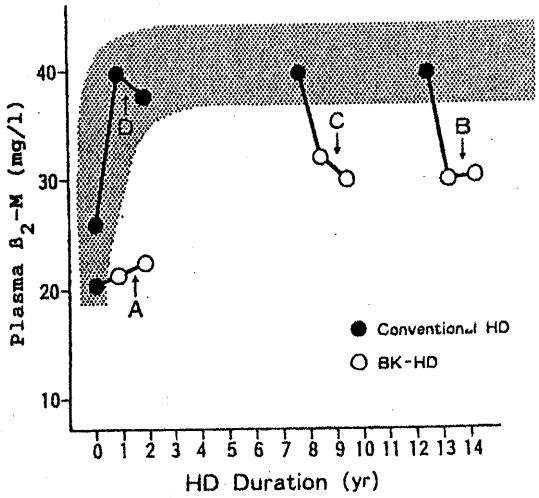

Fig. 5. Changes in plasma β_2-M levels in patients of groups A to D.

Plasma β_2-M values at the time of starting this study were 39.5 ± 10.6 mg/l and 39.5 ± 7.0 mg/l in groups B and C, respectively. When switched from conventional membrane to BK membrane, they were significantly decreased after 1–3 months (31.1 ± 7.4 mg/l in group B, 30.1 ± 7.2 mg/l in group C), although levelling off or plateauing phenomenon was seen in both groups (Fig. 4).

These results are combined in Fig. 5. They suggest strongly that continued use of β_2-M-removable membrane can reduce its plasma concentration significantly, and that the earlier introduction of such a membrane can reduce plasma β_2-M more than in the case of its later introduction.

The change in the pain index is summarised in Fig. 6. The percentage of improved cases was increased as BK membrane haemodialysis duration extended (13% after 6 months, 27% after 12 months, and 39% after 24 months from BK membrane haemodialysis introduction). Furthermore, pair-wise analysis on the change of the pain index was performed, as shown in Fig. 7. Again a statistically significant decrease in the pain index was demonstrated throughout the 2 years clinical evaluation of 41 patients (3.46 ± 0.78 at the time of BK membrane haemodialysis introduction, 1.89 ± 0.96 after 24 months).

Discussion

Since β_2-M has been identified as a major component of amyloid deposits in chronic haemodialysis patients, we measured the plasma concentration of this substance frequently in many uraemic patients before and after the introduction of haemodialysis treatment (Fig. 8). The plasma β_2-M value increased abruptly once GFR decreased to less than 20 ml/min before the introduction

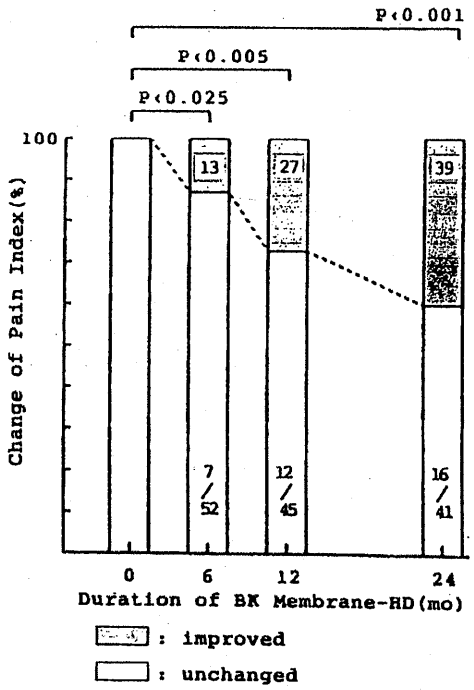

Fig. 6. Time course of changes of pain index during BK membrane haemodialysis. Statistical significance was checked by χ^2 test.

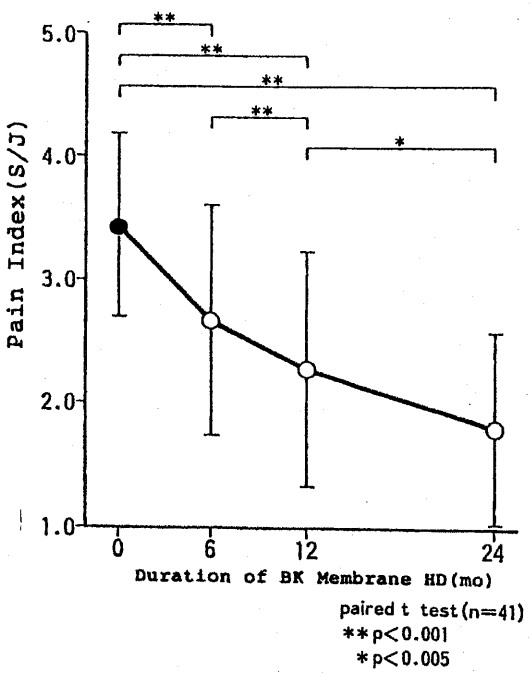

Fig. 7. Changes of pain index during BK membrane haemodialysis. Paired t test was used for pairwise comparison.

of haemodialysis. After starting haemodialysis, all patients revealed extremely elevated scattered plasma β_2-M irrespective of haemodialysis duration. This does

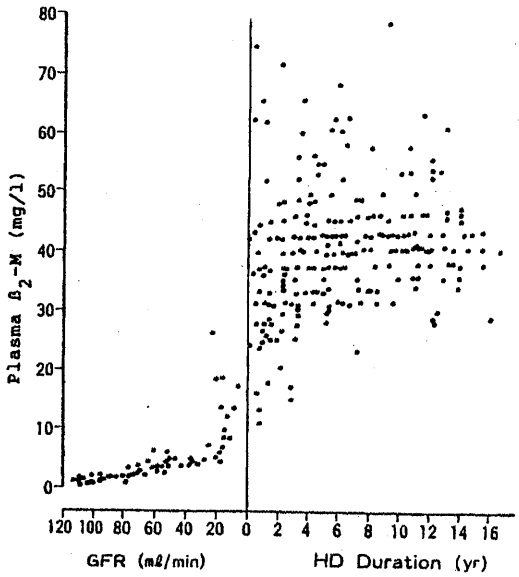

Fig. 8. Plasma levels of β_2-M in patients with chronic renal failure.

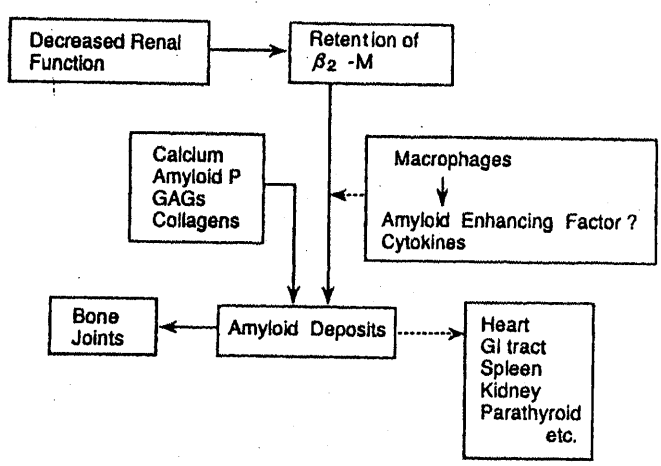

Fig. 9. Hypothesis of pathogenesis of haemodialysis-related amyloidosis.

not seem to correlate with the total duration of haemodialysis treatment.

Our current understanding and hypothesis on the mechanism of haemodialysis-related amyloidosis are shown in Fig. 9. We believe that β_2-M plays a major role in this amyloidogenesis. Although the association of various substances and cytokines in β_2-M amyloid deposits is suggested, it seems essential to try to remove β_2-M as much as possible.

According to the results of this long-term multicentre study, haemodialysis using BK membrane can remove β_2-M from the circulation and decrease its plasma concentration significantly. In addition, the earlier introduction of BK membrane haemodialysis can reduce the plasma concentration of β_2-M more effectively than when introduced after a period of conventional haemodialysis. However, we could not obtain

normal plasma concentration of β_2-M even after 2 years' haemodialysis with BK membrane. This may suggest that there is still a negative balance between production and intermittent removal of β_2-M in chronic haemodialysis patients.

Osteoarthropathy seen in chronic haemodialysis patients is caused not only by β_2-M-related amyloidosis but also by secondary hyperparathyroidism, vitamin D deficiency, or aluminium intoxication. However, it has been discovered recently that amyloid deposition is a major cause of this bone and joint lesion. Amyloid fibrils consisting of β_2-M can be identified in the synovia [12] and bone cyst [3] of long-term haemodialysis patients who complain of joint pains. Persistent decrease of plasma β_2-M concentration, even when it remains greater than that of normal subjects, may reduce its accumulation in the synovia and bone. The continued use of BK membrane clearly improved the joint pain of long-term haemodialysis patients, although it is still uncertain whether deposited β_2-M in the joints can be moved into circulation.

Through our long-term clinical study of BK membrane, two results were demonstrated: significant decreases in plasma β_2-M and in joint pains. At the present time we consider that these favourable results may be due to the removal of β_2-M and probably some other middle molecules by BK membrane. Further study on this subject is required.

Appendix

Niigata Research Programme for β_2-M Removal Membrane

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4. Chuetsu Clinic of Tachikawa General Hospital; Dr Norio Obata.
5. Kitamachi Clinic; Dr Noriaki Kobayashi.
6. Niigataken Kohseiren Chuou General Hospital; Dr Kazuo Kobayashi, Dr Masanosuke Nagao.
7. Kokuho Suibarago Hospital; Dr Kazuya Kawada.
8. Kido Hospital; Dr Shogo Yada, Dr Takashi Ota.
9. Kaetsu Hospital; Dr Kazuyuki Watanabe.
10. Shinrakuen Hospital; Dr Yoshihei Hirasawa, Dr Masashi Suzuki.
11. Ohmori Clinic; Dr Hiroshi Ohmori.
12. Nagaoka Red Cross Hospital; Dr Shoji Miyamura, Dr Kensuke Suzuki.
13. Tsubame Rohsai Hospital; Dr Michiko Shimizu.
14. Katagiri Clinic; Dr Masanori Katagiri.
15. Niigataken Kohseiren Sanjo General Hospital; Dr Akira Kamimura.
16. Tsukanome Clinic; Dr Mamoru Kawauchi.
17. Niigatakenritsu Shibata Hospital; Dr Yoshiaki Miura.
18. Niigatakenritsu Muikamachi Hospital; Dr Hirokazu Sato.
19. Sadogun Kohseiren Sado General Hospital; Dr Masanori Tajiri.
20. Niigataken Kohseiren Kariwagun General Hospital; Dr Noriyuki Homma.
21. Niigatakenritsu Chuou Hospital; Dr Takao Yamakawa, Dr Yuichiro Maruyama.
22. Niigataken Kohseiren Itoigawa Hospital; Dr Yoshiyuki Takano.
23. Ojiya General Hospital; Dr Toshiaki Ikeda.
24. Tokamachi Clinic; Dr Toshiaki Ikeda.
25. Niigataken Kohseiren Joetsu General Hospital; Dr Mitsutoshi Fukagawa.
26. Niigataken Kohseiren Keinan Hospital; Dr Yukihiro Morita.
27. Niigatakenritsu Koide Hospital; Dr Takashi Sato.
28. Niigatakenritsu Yoshida Hospital; Dr Kazuhiko Oohara.

In collaboration with

Keiko Akaoka, Hiroshi Kataoka, Satoko Yamada, Yoshitada Sakai, Tetsunosuke Kunitomo, and Takayuki Takeyama (Toray Industries, Inc.).

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