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Trial use of a polymethylmethacrylate membrane for the removal of free immunoglobulin light chains in dialysis patients

Wataru Oshihara[1], Hirotomo Nagao[2], Hiroshi Megano[3], Jiro Arai[4], Masahumi Koide[2] and Mikihiko Takada[3]

[1]Toray Medical Co., Ltd, Tokyo, Japan, [2]Medical Satellite Iwakura, Aichi, Japan, [3]Medical Satellite Chita, Aichi, Japan and [4]Medical & Biological Laboratories Co., Ltd, Nagano, Japan

Correspondence and offprint requests to: W. Oshihara; E-mail: Wataru_Oshihara@TMC.toray.co.jp

Abstract

Background. Free immunoglobulin light chains (FLCs) accumulate at high levels in the blood of dialysis patients and are likely to cause immunodeficiency during periods of dialysis. Our group examined the blood FLC concentration, κ/λ ratio and rates of FLC removal in different dialysis modes using different dialysis membranes.

Methods. Polymethylmethacrylate (PMMA) membrane (BG-PQ, Toray) under the haemodialysis (HD) condition was used for seven chronic maintenance dialysis patients who had been treated by hemodiafiltration (HDF) (five polyester-polymer alloy (PEPA) and two polysulphone) in a crossover fashion. FLCs in serum were measured with a FREELITE™ Human Kappa Free Kit and Lambda Free Kit prepared by Binding Site, UK (supplied by Medical & Biological Laboratories Co., Ltd, Japan). Each κ-type and λ-type FLC was quantitatively measured with a Dade Behring BNII™. Western blotting was conducted using a goat anti-human κFLC polyclonal antibody.

Results. The κ and λ serum FLCs in HD patients (n = 7) were 157.4 ± 88.9 and 121.9 ± 56.3 mg/L, respectively, and were accumulated in concentrations 4- to 16-fold higher than the concentrations in healthy controls. The κ/λ ratio was included in the reference range (0.26–1.65). In the HD cases dialysed with PMMA membrane, the FLCs were removed mainly by adsorption, leaving only very small quantities in the whole dialysis waste fluid. The ratio between the total removed quantity and albumin leakage ((κ+λ)/Alb) was higher in the PMMA HD group than in the HDF group, and the rate of κFLC removal was higher than the rate of λFLC removal. The serum κFLC in dialysis patients had a multimer structure. In western blotting, the adsorbed κFLC (including multimer structure) detected in the PMMA membrane exceeded that detected in the dialysate with the polysulphone membrane.

Conclusions. Although the FLCs were removed at high rates under the HDF condition, they were also effectively removed by adsorption with the PMMA membrane under the HD condition. It may be possible to effectively remove the FLC multimer with a PMMA membrane *via* adsorption. In future studies on FLC removal in patients with chronic renal failure, it will be necessary to assess not only the quantity of simple removal from the blood but also the qualitative properties, such as the degree to which accumulation and removal rates depend on the κ/λ ratio and multimer structures.

Keywords: haemodialysis; free immunoglobulin light chain; polymethylmethacrylate membrane; polymerization

Introduction

The free molecule of the immunoglobulin light chain (FLC) is also referred to as the 'Bence Jones protein'. This protein is a diagnostic criterion for high M proteinaemia in conditions such as multiple myeloma or primary amyloidosis. A high blood concentration of the protein leads to various protein deposits in the internal organs. The monomer has a molecular weight of 25 kD, and a dimer-to-oligomer transition is observable in the blood of patients with high M proteinaemia. Two molecules, the κ-type and λ-type, are known. The ratio between κFLC and λFLC is thought to be clinically significant.

Cohen *et al*. [1] found that the FLCs are present in higher concentrations in haemodialysis (HD) patients than in healthy subjects, are significantly removed by HD with polymethylmethacrylate (PMMA) membrane and act as inhibitors of leukocyte function and immune function in dialysis patients.

Two papers have recently been published on FLCs in HD patients. The first, by Hutchison *et al*. [2], demonstrated significant abnormalities of serum and urinary polyclonal FLC in patients with Chronic Kidney Disease (CKD). The second, by Hata *et al*. [3], described a significant reduction of FLC multimers and monomers in a patient with primary amyloidosis following adsorptive treatment by the PMMA membrane.

FLCs accumulate at high levels in the blood of dialysis patients and are likely to cause immunodeficiency in HD

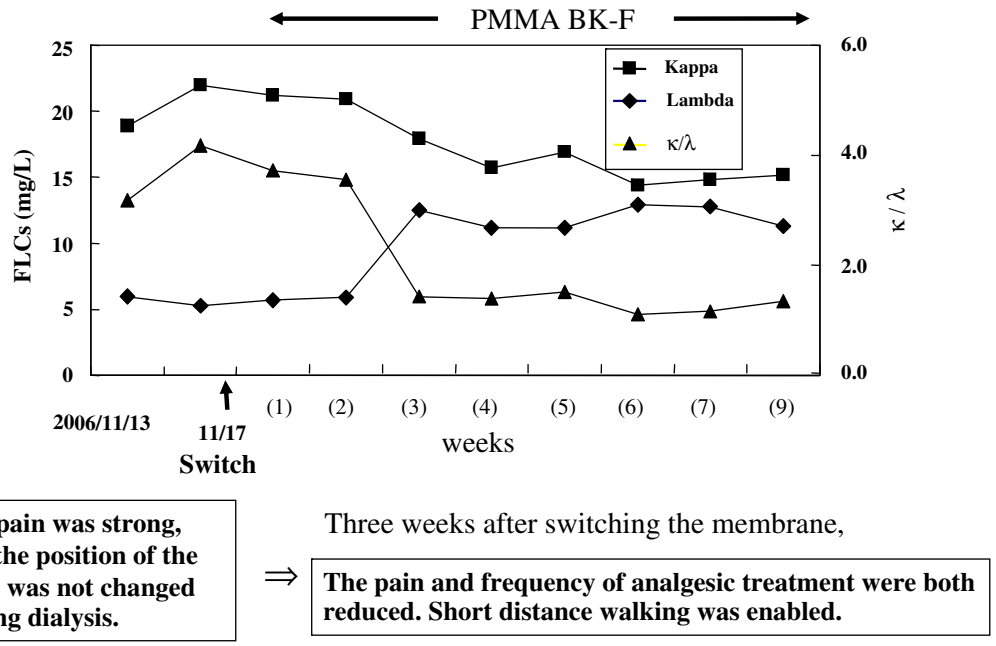

Fig. 1. Case report. The switch to the PMMA membrane resulted in changes in the serum FLC concentration, κ/λ rario, and clinical symptoms.

patients. Our objectives in this study are to report the blood FLC concentration, multimer formation and κ/λ ratio in one HD patient and to assess FLC removal by different dialysis modes using different dialysis membranes in seven dialysis patients.

Case report

The patient was an 85-year-old woman with renal insufficiency. She was diagnosed with multiple myeloma. IgA-κ monoclonal protein was detected in serum by immunoelectrophoresis. Based on her physical condition, she opted against chemotherapy. When she switched to the PMMA dialysis membrane, her clinical symptoms changed. Before the switch, her κ/λ ratio was very high (see the line in Figure 1). She suffered severe pain and had difficulty in changing her body position during the dialysis. However, from 3 weeks after the switch to the PMMA membrane, her pain subsided and the frequency of the analgesic treatment could be decreased. Short distance walking was enabled. The κ/λ ratio fell to within the reference range.

Methods

Patients and dialysis conditions

Seven dialysis patients who had been treated with HDF were switched to HD with the PMMA membrane, and the properties of FLC removal were compared. The patients were six men and one woman (average age, 51.1 ± 18.3 years) who had been on dialysis for an average of 15.3 ± 8.5 years. The underlying diseases were chronic glomerulonephritis (four), SLE (one), purpura-related nephritis (one) and unidentified (one). The dialysis was conducted over a 4-h period at blood flow rates of 150–300 ml/min. HDF modes with PEPA or polysulfone (PS) membrane were switched to HD modes with PMMA membrane in a crossover fashion. Two patients underwent online HDF (post 8-L substitution), one underwent online HDF (pre 40-L substitution) and four underwent push and pull HDF.

Measurement of FLC in serum

FLC in serum or in the extract from the membrane after HD was quantitatively measured using a FREELITE Kit prepared by Binding Site (Birmingham, UK) on a BNII nephelometer manufactured by Dade Behring (Marburg, Germany).

Membrane extract and western blotting

Proteins adsorbed in the PMMA membrane were extracted with 500 ml of 40% acetic acid, neutralized with NaOH and used as specimens. FLCs in patient serum and the extract from the membrane were analysed by western blotting. A 1:500 dilution of patient serum and a membrane extract diluted to the same FLC concentration were fractionated by SDS-PAGE : Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis in a non-reduced condition, and the proteins were fixed on a Poly Vinylidene Di Fluoride (PVDF) film. The κFLC on the film was stained with a goat anti-human κFLC polyclonal antibody (Bethyl, US) and HRP-conjugated second anti-goat IgG monoclonal antibody and then detected by the chemiluminescence method.

Results

Table 1 shows the FLC concentrations in the serum of the seven dialysis patients. The average κFLC and λFLC were 157 and 122 mg/L, respectively. While these levels were high, ranging from 4 to 16 times those of healthy controls, the κ/λ ratio of each patient remained within the reference range (the reference ranges of κFLC, λFLC and the κ/λ ratio were 3.3–19.4 mg/L, 5.7–26.3 mg/L and 0.26–1.65, respectively.) This suggested that the FLC accumulation in dialysis patients is based not on the monoclonal accumulation of FLC molecules but on insufficient catabolism of this protein. No differences in diseases or dialysis histories could be found.

Table 1. FLC concentrations in the serum of the chronic dialysis patients

	Underlying disease	Membrane	Mode	Substitution (L/session)	κFLC before HDF (mg/L)	λFLC before HDF (mg/L)	K/λ
1	CGN	PS 1.6 m^2	P/P HDF	38.2	325.5	219.5	1.5
2	SLE	PEPA 2.1 m^2	P/P HDF	35.5	187.5	162.0	1.2
3	purpura	PEPA 2.1 m^2	P/P HDF	40.5	202.5	151.0	1.3
4	CGN	PEPA 2.1 m^2	P/P HDF	37.7	103.4	74.3	1.4
5	CGN	PS 2.1 m^2	On line HDF	8 (post)	75.8	84.3	0.9
6	CGN	PEPA 2.1 m^2	Online HDF	8 (post)	124.5	77.6	1.6
7	NI	PEPA 2.1 m^2	Online HDF	40 (post)	82.5	84.3	1.0
Mean ± SD					157.4 ± 88.9	121.9 ± 56.3	1.3 ± 0.3

Reference range (Binding Site, UK): K-FLC, 3.3–19.4 mg/L; λ-FLC, 5.7–26.3 mg/L; K/λ, 0.26–1.65.

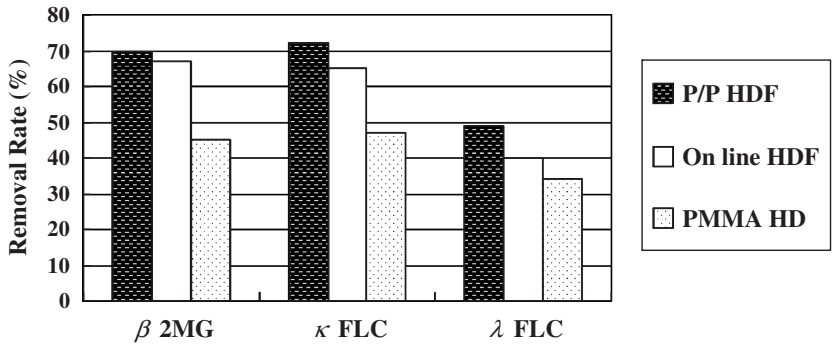

Fig. 2. FLC removal in the different dialysis modes.

Table 2. Removal of low-molecular-weight proteins

Membrane	Mode	Removal amount (mg) estimated			Albumin in dialysate (g/session)	(κ + λ)/albumin (mg/g)
		β2-MG	κFLC	λFLC		
PEPA	P/P	320 ± 76	754 ± 246	330 ± 106	6.8 ± 1.6	158 ± 15
PS or PEPA	Online	240 ± 48	329 ± 130	146 ± 43	3.7 ± 1.5	132 ± 5
PMMA (BG-PQ)	HD	217 ± 52	369 ± 126	182 ± 89	2.2 ± 0.5	269 ± 143

Quantity estimated from the change in blood concentration.

Figure 2 shows the variation in FLC removal from one dialysis mode to another. The HDF mode removed three of the proteins (β2MG, κFLC and λFLC) more effectively than the HD with the PMMA membrane. Importantly, we found that λFLC is harder to remove than κFLC in all dialysis modes tested. It may be that a more polymerized λFLC structure is formed.

The amounts of the three proteins removed are indicated in Table 2. These amounts are estimated from the changes in blood concentration during the HD treatment. The HDF mode not only removed all of the proteins in higher quantities but also increased the levels of albumin loss. The PMMA was effective for FLC removal, as the albumin loss was kept moderate.

Figure 3 shows the polymer structure of the FLCs and the characteristics of FLC removal with the PMMA membrane. Target specimens were the patient serum before and after HD with the PMMA membrane, the elution of adsorbed proteins from the PMMA membrane and the spent dialysate from the polysulphone membrane in HDF mode. As the figure shows, the serum κFLC of one of the dialysis patients had a multimer structure. In addition, more of the κFLC dimers were removed in the dialysis with the PMMA membrane. The amount of κFLC adsorbed by the PMMA membrane exceeded the amount of κFLC in the spent dialysate from the dialysis with the polysulphone membrane.

Figure 4 shows the changes of four low-molecular-weight proteins in six patients undergoing dialysis for a period of 6 months. Treatments with PMMA membrane under the HD condition were conducted for the first 3 months; then, the patients were switched to online HDF (16 L of substitution, post dilution) with the same membrane for the second 3 months. The decreases of β2MG and α1MG were smaller than the decreases of κFLC and λFLC. The blood FLC concentration is report-

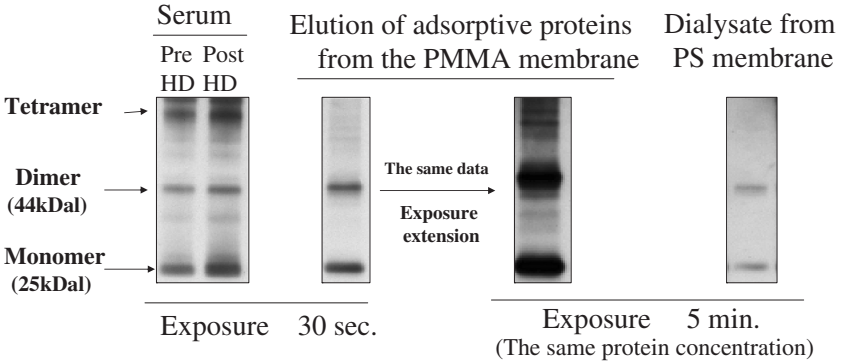

Fig. 3. Polymer structure of the κFLC and removal characteristics in haemodialysis with the PMMA membrane.

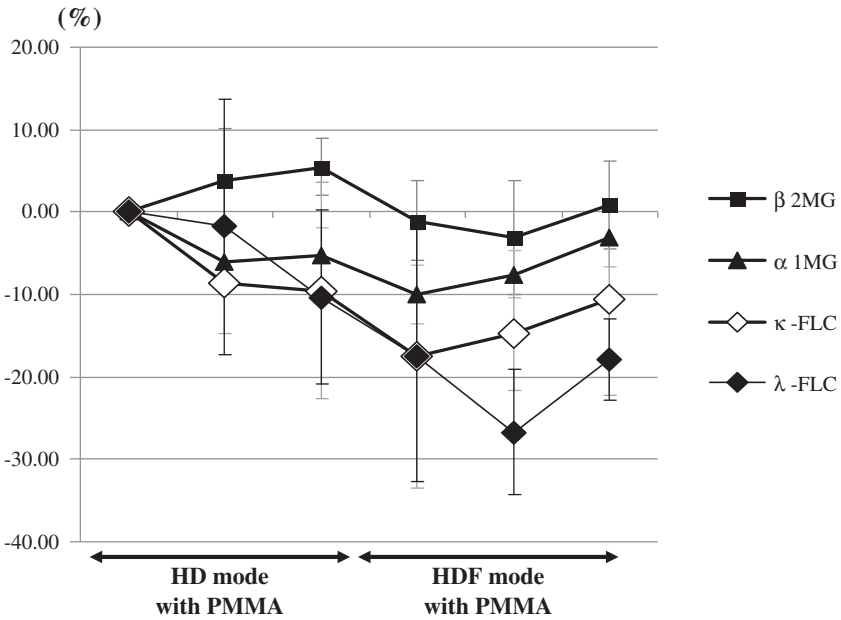

Fig. 4. Changes of low molecular weight proteins in HD or HDF mode with the PMMA membrane.

edly difficult to reduce over the long term [2]. The FLC blood concentration fell to some extent by a single blood purification, but it rose by the time the next treatment commenced. Although the removal rate was maximized in the HDF treatment with the polysulphone-based membrane, a decrease of the FLC blood concentration during the middle period could not be found (data not shown). The blood FLC concentration in the middle period was only observably reduced in the patients undergoing HD or HDF treatment with the PMMA membrane.

Discussion

Solling [4] and Wakasugi *et al*. [5] reported elevated FLC concentrations in the serum of patients with chronic renal failure. More recently, Hutchison corroborated this result [2]. In the study by Solling, CRF patients had a high κ/λ ratio (1.9) and clearly elevated serum FLC concentrations compared with the healthy subjects. κFLC, a protein easily excreted *via* the kidney in healthy subjects because of the difference in the degree of κFLC and λFLC polymerization, is only minimally excreted in CRF patients. This accounts for the increase in the κ/λ ratio in our patients. Hutchison *et al*. [6] determined that the reference range for the κ/λ ratio in CKD patients should be raised from 0.26 to 1.65 and, the range in healthy subjects, to 0.37–3.1.

The κ/λ in our seven HD patients ranged from 0.9 to 1.6, and λFLC was harder to remove than κFLC in all of the dialysis modes and membranes investigated. It may be that a more polymerized λFLC structure forms. In our western blotting analysis, the dimer and tetramer of κFLC were abundant in the serum of the dialysis patients. In future studies, we plan to confirm both the presence and multimer structure of λFLC by western blotting.

The presence of both multimer and monomer in extracts from PMMA membrane indicates that κFLC is adsorbed onto the membrane in all of its molecular states. The effective removal of FLC multimer with the PMMA membrane may take place *via* the mechanism of adsorption rather than diffusion or filtration. If this is so, we can expect the FLC concentration to fall in HD patients dialysed with the PMMA membrane.

A recent study has found evidence that monoclonal FLC is toxic. Monoclonal FLC seems to promote proximal tubular injury [7], epithelial–mesangial transition [8] and other processes. In the studies by Cohen *et al*. [2,9,10], the uraemic toxicity of polyclonal FLC mediated the reduction of neutrophil function. Hutchison *et al*. [2] showed that urinary polyclonal FLC concentrations vary according to the type of renal disease, CKD stage and albuminuria. These data suggest that polyclonal FLC contributes to progressive renal injury and systemic inflammation in patients with CKD.

Through the choice of the dialysis mode and dialysis membrane, the FLCs could be removed with high efficiency. FLC removal by the PMMA membrane was the most effective. The serum κFLC in the dialysis patients had a multimer structure. Henceforth, it will also be necessary to examine the multimer structure in λFLC, a property known to be aetiologically relevant in multiple myeloma, in dialysis patients.

The removal of FLCs from the blood of patients with chronic renal failure should be evaluated not only by the quantity of simple removal but also by qualitative properties such as the levels of accumulation and removal in association with the κ/λ ratio and multimer structures.

Conflict of interest statement. Wataru Oshihara is an employee of Toray Medical Co., Ltd.

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