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Adsorption of Complement, Cytokines, and Proteins by Different Dialysis Membrane Materials: Evaluation by Confocal Laser Scanning Fluorescence Microscopy

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Abstract: The membranes tested in the present study were cellulose triacetate (CTA), polymethylmethacrylate (PMMA), and polyacrylonitrile (PAN). The adsorption by each membrane of albumin, IgG, C3a, interleukin-1β (IL-1β), interleukin-6 (IL-6), human neutrophil elastase (HNE), and tumor necrosis factor α (TNFα) was examined and semiquantitatively graded by confocal laser scanning fluorescence microscopy (CLSFM). After clinical use the dialyzers were treated with antibodies for these proteins and cytokines. Then the samples were incubated with fluorescein isothiocyanate-labeled anti-IgG antibody and observed by CLSFM. The changes in the blood levels of C3a and cytokines were also studied. In the CTA membrane, the adsorption of these substances, except for albumin and HNE, was less than in the synthetic membranes. The PAN membrane revealed the most abundant adsorption, especially for IL-1β, IL-6, and TNFα. Although a marked elevation of C3a in the blood was observed in the CTA membrane, considerable adsorption was evident in the PMMA and the PAN membranes. Because the changes in the blood levels could be affected by membrane adsorption, both the blood levels and the adsorption of the biocompatibility parameters should be evaluated when membrane biocompatibility is discussed. **Key Words:** Confocal laser scanning fluroescence microscopy—Dialysis membrane—Biocompatibility—Cytokine—C3a—Adsorption.

The confocal laser scanning fluorescence microscopy (CLSFM) technique is a highly specific and sensitive method used to demonstrate membrane adsorption of a certain substance. Not only the existence, but also the distribution of the adsorbed substance in the membrane section can be examined. Therefore, this method is quite useful for investigations of the adsorptive characteristics of dialysis membranes.

The biocompatibility of dialysis membranes is a defined issue in terms of achieving safer hemodialysis treatments (1). It is usually evaluated in vivo by means of the measurement of the blood levels of such biocompatibility parameters as complements and cytokines (1–8). However, because these substances could have been adsorbed by the membranes, even when the elevations in the blood were not significant, substantial amounts of these substances might have been generated. Therefore, in the present study, we utilized CLSFM to investigate the membrane adsorption of the biocompatibility markers as well as other serum proteins.

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MATERIALS AND METHODS

Dialysis membranes

The membrane materials tested were cellulose triacetate (CTA), polymethylmethacrylate (PMMA), and polyacrylonitrile (PAN). The product names were the FB-150U (Nipro Co., Ltd., Osaka, Japan), BK-1.6P (Toray Medical Co., Ltd., Tokyo, Japan), and PAN-17DX (Asahi Medical Co., Tokyo, Japan) membranes, respectively. The membrane areas, mean pore radii, and membrane thicknesses of the 3 dialyzers are shown in Table 1.

Hemodialysis treatment

Hemodialysis was performed using a dialysis machine, the DCS-22B, from Nikkiso Co., Ltd. (Tokyo, Japan) with a single pass system and controlled ultrafiltration. The treatments were performed for 4 h with

TABLE 1. *Three different dialyzers used in this study (membrane area, mean pore radius, and membrane thickness from catalogues)*

Membrane	Area (m^2)	Pore radius (Å)	Thickness (μm)
FB-150U (CTA)	1.5	70	15
BK-1.6P (PMMA)	1.6	80	30
PAN-17DX (PAN)	1.7	80	35

heparin for anticoagulation, a blood flow rate of 200 ml/min, and a dialysate flow rate of 500 ml/min.

Study strategy

The dialysis membranes were used for 3 patients with end-stage renal disease (2 males and 1 female) undergoing maintenance hemodialysis (3 times/week). Each patient had dialysis with each membrane (CTA, PMMA, and then PAN) 12 times. At the 10th exposure to a membrane, studies on both the membrane adsorption and blood levels were performed.

CLSFM technique

After clinical use the hollow fibers were washed with 500 ml of saline, and the membrane samples (approximately 2 cm long) were taken from the middle of the fibers. After having been fixed in 2% paraformaldehyde for 4 h, samples were rinsed in phosphate buffered saline, then cut into 0.1 mm thick slices. The membrane slices were treated with antibodies for human albumin, IgG, β_2 microglobulin (β_2-MG), C3a, interleukin-1β (IL-1β), interleukin-6 (IL-6), human neutrophil elastase (HNE), and tumor necrosis factor α (TNFα). Then, they were treated with fluorescein isothiocyanate-labeled anti-IgG antibody. Finally, the samples were observed under a confocal laser scanning fluorescence microscope from Olympus Optical Company (Tokyo, Japan). The intensity of the fluorescence was graded in terms of 5 colors.

Measurement of the blood parameters

The blood levels of the biocompatibility parameters were measured by a commercial laboratory (SRL Co., Tokyo, Japan). Blood was drawn from both the inlet and outlet of the dialyzer 15 min from the beginning of each treatment. The C3a, HNE, IL-1β, IL-6, and TNFα levels were determined using a radioimmunoassay kit from Amersham International PLC. (Buckinghamshire, England), and enzyme immunoassay kit from Sanwa Chemical Laboratory Co. (Nagoya, Japan), a radioimmunoassay kit from BioSource Europe S.A. (Fleurus, Belgium), a chemiluminescent enzyme immunoassay kit from Fujirebio Co. (Tokyo, Japan), and an enzyme-linked immunosorbent assay kit from Japan Antibody Laboratory Co. (Takasaki, Japan), respectively.

RESULTS

The fluorescence intensity observed by CLSFM is summarized in Table 2. In the CTA, although strong fluorescence for albumin and HNE was observed, the adsorption of other substances was less than in the synthetic membranes. In the PMMA, fluorescence was generally more intense than in the CTA. The PAN showed the strongest fluorescence, especially for IL-1β, IL-6, and TNFα.

Representative results of the CLSFM study are shown in Figs. 1 and 2. Figure 1 shows microscopic views of the membrane sections demonstrating fluorescence for C3a. Weak fluorescence for C3a was observed near the inner surface of the CTA. In the PMMA, fluorescence was seen near both the inner and outer surfaces. The PAN showed stronger fluorescence mainly near both surfaces.

Figure 2 shows the fluorescence indicating TNFα

TABLE 2. *Summary of the CLSFM observation*

Membrane	Section of membrane (by thirds)	Alb	IgG	β2-MG	C3a	IL-1β	IL-6	HNE	TNF-α
CTA	inner	++	+	±	±	±	−	+	±
	middle	−	−	−	−	−	−	±	−
	outer	+	±	±	−	−	−	+	±
PMMA	inner	−	++	−	±	−	−	+	−
	middle	−	±	±	−	−	−	−	−
	outer	±	++	+	+	+	±	++	+
PAN	inner	+++	++	+++	+	+++	+	++	+++
	middle	+++	+++	±	±	+	+	++	++
	outer	++	++	++	++	+++	++	++	+++

Membrane samples from three patients were observed under a CLSFM. CLSFM scores were almost identical even though patients were different. Fluorescence intensity of the inner ⅓, middle ⅓ and outer ⅓ of each membrane section is expressed as −, ±, +, ++, +++ according to the CLSFM score.

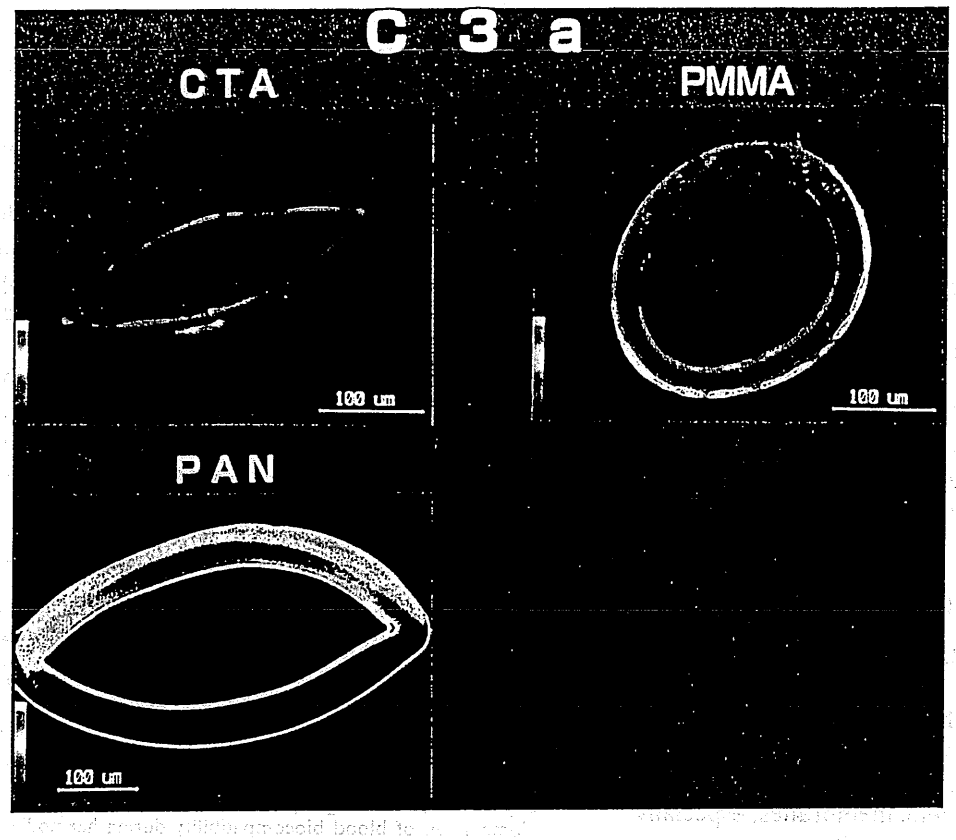

FIG. 1. To determine the fluorescence for C3a, membrane sections of CTA, PMMA, and PAN were observed with CLSFM. Fluorescence indicates C3a adsorption. Representative results from 3 patients are shown.

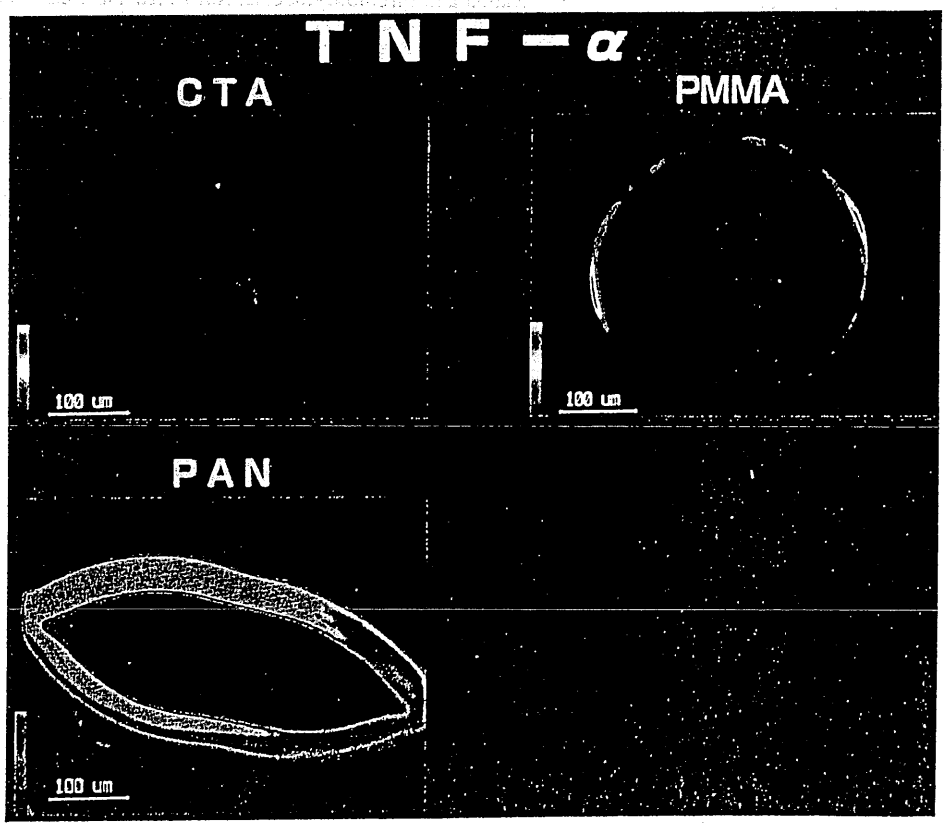

FIG. 2. To evaluate the fluorescence for TNFα, membrane sections of CTA, PMMA, and PAN were observed with CLSFM. Fluorescence indicates TNFα adsorption. Representative results from 3 patients are shown.

adsorption. Fluorescence was hardly seen in the CTA section. The PMMA section showed weak fluorescence near the outer surface. Strong fluorescence for TNFα was observed throughout the section of the PAN membrane.

The blood levels of these parameters were also measured at both the inlets and outlets of the dialyzers. The increments in the level of C3a at 15 min after dialysis start with the CTA, PMMA, and PAN membranes were 1.89 ± 0.81, 1.12 ± 0.44, and 1.16 ± 0.13 (mean ± SD of outlet/inlet ratio, n = 3), respectively. The IL-6 concentrations were not affected by these membranes. The changes in the HNE concentrations were small in comparison to the strong fluorescence observed on these membranes. Most of the blood levels of IL-1β and TNFα were below the sensitivity limits.

DISCUSSION

It is known that synthetic membrane materials such as PMMA and PAN adsorb serum proteins like albumin, IgG, and β_2-MG. However, our CLSFM study demonstrated not only the adsorption of these proteins, but also the abundant adsorption of C3a and cytokines in the synthetic membranes, especially the PAN membrane. If the significant removal of toxic cytokines, TNFα, for example, were achieved using these synthetic membranes in patients with acute renal failure related to sepsis and/or multiorgan failure, the adsorptive characteristics of these membranes could be beneficial and might partially account for the earlier recovery of acute renal failure when patiens have been dialyzed with synthetic membranes (1).

In the present study, the area of the CTA membrane was 1.5 m^2, which was smaller than of the PMMA or the PAN membrane. The mean pore radius was almost the same in the 3 membranes. Therefore, it is impossible to explain the different adsorptive characteristics by the surface area or pore size. Differences in the adsorption appear to come from other factors such as the electrical charge.

Although the PMMA and the PAN caused less significant changes in the blood levels of C3a, abundant adsorption has been demonstrated in these synthetic membranes. These results might suggest that a substantial amount of C3a could be generated in these synthetic hollow-fiber membranes. Therefore, when membrane biocompatibility is discussed, both the blood levels and the adsorption of the biocompatibility factors should be evaluated.

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