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COMPARISON OF SERUM PROTEIN REMOVAL IN HAEMODIALYSIS THERAPY BY PARTLY PROTEIN-PERMEABLE HAEMODIALYSERS

WEN-YANG CHIU and KAZUMASA YAMADA

Nagoya University Branch Hospital, 1-1-20, Daiko-cho, Higashi-ku, Nagoya 461 (Japan)

and

AKIRA SAITO, HIROSHI OGAWA, TOYOMI TAKAGI and TAI GI CHUNG*

Bio-Dynamics Research Institute, 1-3-2, Tamamizu-cho, Mizuho-ku, Nagoya 467 (Japan)

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SUMMARY

Partly protein-permeable haemodialysers were evaluated during haemodialysis therapy for removal of serum low-molecular-mass proteins (10 000–76 000) in patients with chronic renal failure. The six haemodialyser membranes used were cuproammonium rayon (CL-S12W), cellulose acetate (Duo-Flux HP and FF-22), ethylene–vinyl alcohol copolymer (KF-101-15), polyacrylonitrile (H12-2400S) and polymethyl methacrylate (BK2.0H). The analysis was carried out by gel permeation chromatography, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis (2-DE). The removal of ten serum proteins per haemodialysis therapy was also carried out by the immunodiffusion method. The protein removal in each haemodialyser is qualitatively comparable to that obtained by SDS-PAGE and 2-DE. β_2-Microglobulin in the haemodialysate obtained with BK2.0H was removed to a lesser extent than that from the other haemodialysers, which seems to be the reason for its adsorption in the BK2.0H haemodialyser, which contains a polymethyl methacrylate membrane. The amount of serum protein excretion during haemodiafiltration treatment using the partly protein-permeable haemodialysers decreased in the order KF-101-15C > BK2.0H > CL-S12W > Duo-Flux HP > FF-22 > H12-2400S.

INTRODUCTION

To date, haemodialysis therapy has been carried out mainly to remove low-molecular-mass compounds from patients with chronic renal failure, but has not been concerned with the removal of patients' serum proteins. Elevated amounts of low-molecular-mass proteins (M_r 10 000–76 000) have been demonstrated in haemodialysis patients [1,2]. More recently, β_2-microglobulin (M_r 11 800) has been reported to be a uraemic toxin-forming amyloid fibril in haemodialysis patients with carpal tunnel syndrome [3]. Several other complications, such as

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anaemia and bone pain, have also occurred in long-term haemodialysis patients. Clinical therapy resulting in a greater clearance of low-molecular-mass proteins, such as continuous ambulatory peritoneal dialysis [4,5] and partly protein-permeating haemodiafiltration [6], has been effective in alleviating the complications. Recently, there has been more use of haemodialysis with a haemodialyser with a higher cut-off point than conventional haemodialysers, giving a better removal of medium-range molecules (M_r 500–5000) and low-molecular-mass proteins.

In this study, we evaluated the performance of commercially available partly protein-permeable haemodialysers by analysing patients' haemodialysates. Haemodialysers prepared from cuproammonium rayon (CU; CL-S12W), cellulose acetate (CA; Duo-Flux HP and FF-22), ethylene–vinyl alcohol copolymer (EVAL; KF-101-15C), polyacrylonitrile (PAN; H12-2400S) and polymethyl methacrylate) (PMMA; BK2.0H) were examined by gel permeation chromatography (GPC), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE) and a single radial immunodiffusion method.

EXPERIMENTAL

Chemicals

The following hollow-fibre cartridges of artificial kidney were examined for removal of serum proteins: KF-101-15C (Kuraray, Osaka, Japan), BK2.0H and B1-L (Toray, Tokyo, Japan), CL-S12W (Terumo, Tokyo, Japan), Duo-Flux HP (CD Medical, Miami, FL, U.S.A.), H12-2400S (Hospal, Paris, France) and FF-22 (Teijin, Tokyo, Japan) (Table I). The concentrations of the following proteins were measured by a single radial immunodiffusion technique [7] using commercial partigen plates (Behringwerke, Marburg, F.R.G.): retinol binding protein,

TABLE I

SPECIFICITY OF PROTEIN-PERMEABLE HAEMODIALYSERS

Haemodialyser	Material*	I.D. (μm)	Wall thickness (μm)	Surface area (m^2)	Sterilization method**
KF-101-15C	EVAL	200	32	1.5	EOG
BK2.0H	PMMA	245	32	2.0	γ-Rays
CL-S12W	CU	200	23	1.2	Autoclave
Duo-Flux HP	CA	215	30	1.6	EOG
FF-22	CA	205	21	2.3	EOG
H12-2400S	PAN	Plate type	22	1.0	EOG
B1-L***	PMMA	260	30	2.1	γ-Rays

*EVAL = ethylene–vinyl alcohol copolymer; PMMA = polymethyl methacrylate; CU = cuproammonium rayon; CA = cellulose acetate; PAN = polyacrylonitrile

**EOG = ethylene oxide gas.

***B1-L = conventional dialyser.

α_1-acid glycoprotein, β_2-glycoprotein I, α_2-HS glycoprotein, α_1-antitrypsin, prealbumin, haemopexin, albumin and transferrin. β_2-Microglobulin was determined by radioimmunoassay. Acrylamide, N,N-methylenebisacrylamide, N,N,N′,N′-tetramethylenediamine (all special grade for electrophoresis), Tris base, glycine and ammonium persulphate were obtained from Wako (Osaka, Japan) and Ampholines (pH 3.5–10 and 3.5–5) from LKB (Bromma, Sweden). Seamless cellulose tubing (size 36/32) was purchased from Union Carbide (New York, NY, U.S.A.). All other chemicals were of analytical-reagent grade.

Gel permeation chromatography

A Twinkle high-performance liquid chromatograph and a UVIDEC-100-II UV detector (Japan Spectroscopic, Tokyo, Japan) were used, equipped with a Rheodyne 7125 sample injection valve (100-μl loop). An Asahipak GS-320 (particle size 9 μm) column (500 mm × 7.6 mm I.D.) was obtained from Asahikasei (Tokyo, Japan). The column temperature was ambient (20–25°C). The mobile phase was 0.1 *M* phosphate buffer (pH 7.0) containing 0.3 *M* sodium chloride at a flow-rate of 0.7 ml/min. Detection was carried out at 254 nm.

Gel electrophoresis

2-DE under non-denaturing conditions was carried out according to the previous method [8]. Briefly, one-dimensional isoelectric focusing was carried out on a pre-cast Ampholine 4% polyacrylamide gel column (80 mm × 3.5 mm I.D.) with a pH range of 3.5–10. Electrophoresis was operated at 2 mA constant current per gel column for 8 min and then at 220 V constant voltage for 3 h at 4°C. Polyacrylamide gradient (4–17%) slab gels (82 mm × 82 mm × 2.7 mm) were prepared and 2-DE was performed with the non-denaturing buffer. The non-equilibrated isoelectric focusing gels were applied directly to the top of the slab gels and electrophoresed at 30 mA per gel for 3 h at 4°C. After eletrophoresis, the gels were stained overnight in 0.025% Coomassie Brilliant Blue R-250 in 7% methanol and destained in 7% acetic acid by shaking at room temperature. SDS-PAGE was performed by the method described by Laemmli [9] in a polyacrylamide gradient (8–18%, w/v) slab gel (20 cm × 20 cm) containing 1% SDS. SDS-PAGE was carried out at 10 mA for 12 h at room temperature. Sample solutions were denatured in 1% SDS and 6 *M* urea without reductant at 100°C for 5 min. Samples containing 600 μg of protein were applied to the gels. The gels were stained with 0.025% Coomassie Brilliant Blue R-250 in 7% (v/v) acetic acid by shaking at room temperature. These gels were photographed with a 35-mm camera using Fuji Minicopy II film.

Samples

Ultrafiltrates and haemodialysates were obtained from haemodialysis patients in Shinseikai Dai-Ichi Hospital who were undergoing therapeutic haemodialysis (HD) and haemodiafiltration (HDF) with use of a protein-permeable artificial kidney [10]. The HDF treatment of the twelve patients was carried out for 4–5 h three times a week, using the above-mentioned haemodialysers in a pre-dilution method. The blood flow-rate and dialysate flow-rate were 180–200 ml/min and

500 ml/min, respectively. A volume of 100 ml of HDF dialysate from a patient was dialysed against 0.01 *M* potassium phosphate buffer (pH 7.0) using a cellulose tube and lyophilized. The material was dissolved in the same buffer (1 ml) and the proteins were determined. Conversely, 1 l of ultrafiltrate was obtained from a patient by the extracorporeal ultrafiltration method [11] and the ultrafiltrate was used for analysis without further concentration.

RESULTS

Partly protein-permeable haemodialysers made of CU, CA, EVAL, PAN and PMMA were evaluated by determining the serum proteins in haemodialysate from the clinical treatment of the patients. Fig. 1 shows GPC patterns of the ultrafiltrates: (A) CU membrane (CL-S12W), (B) CA membrane (Duo-Flux HP) and (C) EVAL membrane (KF-101-15C). Although the samples differed from patient to patient, the chromatographic patterns were similar, and the protein fraction and low-molecular-mass compounds such as creatinine and uric acid were separated. Peak a was a protein-eluted fraction. In comparison with these chromatograms, the areas of peak a decreased in the order KF-101-15C > CL-S12W > Duo-Flux HP, which reflects a higher cut-off point of the haemodialysers. The elution patterns of the low-molecular-mass range (peaks b–d) were not closely related to the haemodialyser cut-off point, but revealed the properties of the sample solution.

Fig. 2 shows the SDS-PAGE patterns of each blood ultrafiltrate. Low-molecular-mass proteins were separated. Sample 4 from KF-101-15C showed separate protein bands containing higher-molecular-mass proteins ($M_r > 94\,000$). There was no differrence in the SDS patterns between Duo-Flux HP (sample 2) and CL-S12W (sample 3). With sample 1 from BK2.0H two bands, which are the fastest moving β_2-microglobulin and the band around M_r 20 000, were not detected.

Fig. 3 shows the SDS-PAGE pattern of haemodialysates obtained from HDF therapy using the haemodialysers. The samples (2, KF-101-15C; 3, FF-22; 4,

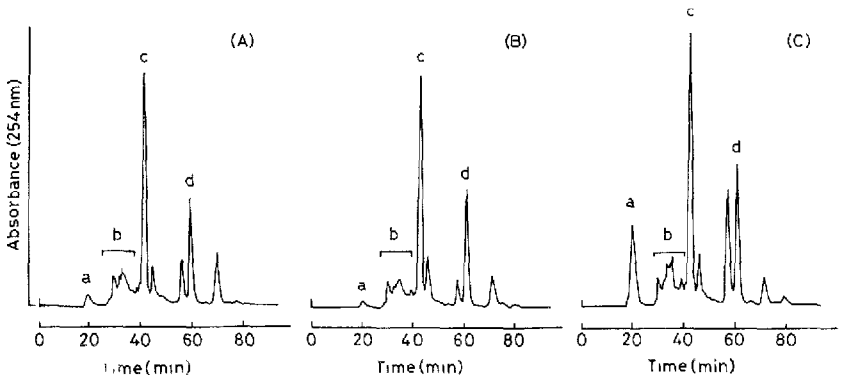
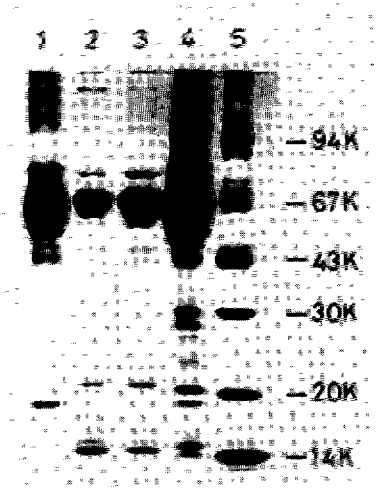


Fig 1. Chromatograms of the blood ultrafiltrates obtained from haemodialysis patients by protein-permeable haemodialysers. Conditions as described under *Gel permeation chromatography*. (A) CL-S12W; (B) Duo-Flux HP; (C) KF-101-15C. For abbreviations a–d, see Results.



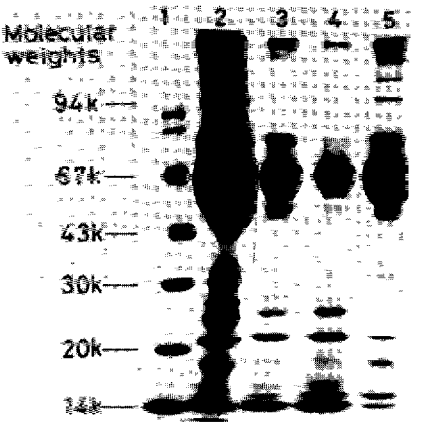


Fig. 2. SDS-PAGE of the blood ultrafiltrates obtained from haemodialysis patients by protein-permeable haemodialysers (1) BK2.0H; (2) Duo-Flux HP; (3) CL-S12W; (4) KF-101-15C; (5) marker proteins: phosphorylase B (M_r 94 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), soybean trypsin inhibitor (M_r 20 000) and α-lactalbumin (M_r 14 400).

Fig. 3. SDS-PAGE of the 100-fold concentrated haemodialysates obtained from haemodialysis patients by protein-permeable haemodialysers. (1) Marker proteins (see Fig. 2); (2) KF-101-15C; (3) FF-22; (4) Duo-Flux HP; (5) BK2.0H.

DuoFlux HP; and 5, BK2.0H) were concentrated 100-fold and analysed. Many low-molecular-mass proteins ranging from M_r 10 000 to 67 000 were removed by this clinical treatment. In comparison with the SDS-PAGE patterns, some protein bands increased, in the order KF-101-15C > FF-22 > BK2.0H > Duo-Flux HP.

Fig. 4 shows the 2-DE patterns of the blood ultrafiltrates: KF-101-15C, approximately thirty spots of proteins, including those from β_2-microglobulin to IgG (M_r 160 000), being observed; CL-S12W and FF-22, ten spots being observed, including mainly transferrin (M_r 76 000) and albumin (M_r 66 000); Duo-Flux HP and H12-2400S, three spots, mainly β_2-microglobulin and acidic albumin, being observed; and BK2.0H, where β_2-microglobulin did not appear. The removal of serum proteins during HDF treatment with the haemodialyser is shown in Table II. On HDF treatment with the H12-2400S haemodialyser, only three proteins (albumin, retinol binding protein and β_2-microglobulin) were detected in the haemodialysate: albumin, 42 ± 34 mg; retinol-binding protein, 47.4 ± 7.9 mg; and β_2-microglobulin, 131.6 ± 40.8 mg.

DISCUSSION

The serum levels of low-molecular-mass proteins (M_r 10 000–76 000) in haemodialysis patients were measured and found to be strikingly higher than the normal levels [1,2]. The pathophysiological effect caused by accumulation of the

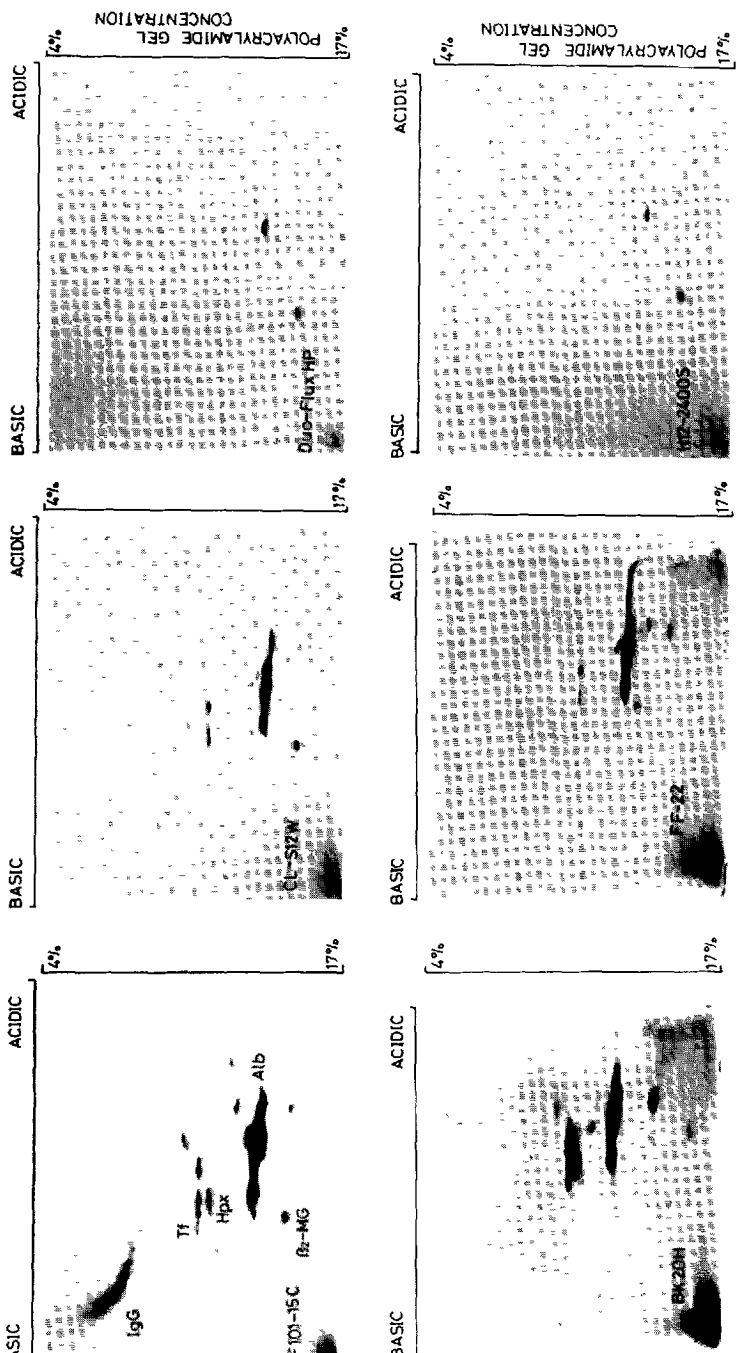


Fig. 4. Two-dimensional electrophoretic separation of the serum proteins in the ultrafiltrates obtained from haemodialysis patients by protein-permeable haemodialysers. IgG = immunoglobulin G; Tf = transferrin; Alb = albumin; Hpx = haemopexin; β_2-MG = β_2-microglobulin.

TABLE II

REMOVAL OF SERUM PROTEINS DURING HAEMODIAFILTRATION WITH PROTEIN-PERMEABLE HAEMODIALYSERS

N.D. = not detected; N M. = not measured.

Serum protein	Amount (mg per 150 l haemodialysate)					
	KF-101-15C ($n=6$)	BK2.0H ($n=3$)	CL-S12W ($n=6$)	Duo-Flux HP ($n=4$)	FF-22 ($n=2$)	B1-L ($n=3$)
Transferrin	516.3 ± 392.7	336.7 ± 173.0	264.0 ± 98.7	144.0 ± 62.0	55.5 ± 10.5	N.D.
Albumin	9957.0 ± 2780.0	6536.0 ± 2189.0	3378.0 ± 1279.0	1395.0 ± 307.0	1305.0 ± 45.0	N.D.
Haemopexin	197.7 ± 95.0	303.5 ± 200.6	130.3 ± 31.5	49.5 ± 23.5	26.3 ± 10.1	N.D.
Prealbumin	146.4 ± 111.6	107.9 ± 32.4	28.7 ± 9.9	18.8 ± 3.8	43.2 ± 13.2	N.D.
α_1-Antitrypsin	1044.1 ± 337.1	461.8 ± 41.8	331.3 ± 97.4	203.5 ± 102.8	152.1 ± 35.1	N.D.
α_2-HS glycoprotein	154.2 ± 65.2	57.3 ± 31.2	N.D.	N.D.	N.D.	N.D.
α_1-Acid glycoprotein	627.2 ± 354.4	334.4 ± 45.8	240.0 ± 61.7	90.0 ± 24.3	62.1 ± 12.9	N.D.
β_2-Glycoprotein I	107.1 ± 28.6	N.D.	30.7 ± 4.9	32.0 ± 13.5	27.5 ± 13.1	N.D.
Retinol binding protein	167 5 ± 45.3	59.8 ± 27.8	78.8 ± 15.8	89 5 ± 25.4	70.3 ± 1.0	N.D.
β_2-Microglobulin	145.2 ± 39.7	N.M.	147.3 ± 51.6	117.0 ± 40.1	N.M.	N.D.

serum proteins and their dynamics during the haemodialysis therapy have been little discussed. For example, the serum level of β_2-microglobulin in the patients is 10–50 times higher than that in normal subjects, and recently β_2-microglobulin-associated amyloid deposit in haemodialysis patients, as one of the uraemic toxins, has been reported [12,13]. This is clearly due to the low removal rates of low-molecular-mass proteins by conventional hollow-fibre kidneys. Haemodialysis using a haemodialyser with a higher-molecular-mass cut-off than a conventional haemodialyser has attracted interest in recent years.

In this work, we evaluated six protein-permeable haemodialysers and a conventional B1-L haemodialyser by determining the concentrations of proteins in the haemodialysates during clinical treatment. Using SDS-PAGE and 2-DE, low-molecular-mass proteins were separated into many bands, which could be easily explained in terms of the performance of each haemodialyser.

Because the KF-101-15C was set at a higher sieving coefficient, in addition to low-molecular-mass proteins such as β_2-microglobulin and retinol-binding protein, albumin and IgG were also substantially removed. The removal ratios of albumin by CL-S12W and Duo-Flux HP were 1/2–1/3 and 1/5–1/7, respectively, compared with those given by KF-101-15C and BK2.0H haemodialysers. Using CL-S12W and Duo-Flux HP, the clearance of low-molecular-mass compounds, such as creatinine and uric acid, was superior to that with KF-101-15C. The clearance with Duo-Flux HP per unit area was the same as that with BK2.0H, but that with CL-S12W was superior to that with BK2.0H (data not shown). As the β_2-microglobulin sieving coefficient of H12-2400S resembled that of Duo-Flux HP and there was little loss of albumin, it was possible to use long-term therapy clinically. In the BK2.0H ultrafiltrate, the β_2-microglobulin band was not observed using SDS-PAGE and 2-DE, owing to the adsorption of serum β_2-microglobulin by the PMMA membrane [14]. The removal of β_2-microglobulin was 100–150 mg per HDF therapy, but it would be necessary to establish the suitable removal of β_2-microglobulin. α_2-HS glycoprotein was not detected in the haemodialysates from cellulose membranes (Duo-Flux HP, CL-S12W and FF-22). It is still unclear whether α_2-HS glycoprotein is adsorbed by cellulose haemodialysers.

Because low-molecular-mass proteins are generally filtered in the glomeruli and taken up by proximal tubular cells from the ultrafiltrate via endocytosis, it will be important to develop a haemodialyser to reduce patients' serum protein levels to normal levels. In this study of haemodialysers, cellulose membranes (CL-S12W and Duo-Flux HP) were found to have a sharper cut-off for low-molecular-mass proteins than the other synthetic membranes, which is desirable for successful haemodialysis therapy.

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