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Original Article

Effect of dialysis on serum/plasma levels of free immunoglobulin light chains in end-stage renal disease patients

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Abstract

Background. Free immunoglobulin light chains (FLCs) have previously been shown to be uraemic toxins. In this work we investigated the effect of haemodialysis and haemodiafiltration on the level of FLCs in serum/plasma of uraemic patients.

Methods. Serum/plasma proteins were separated by non-reducing SDS–PAGE and transferred to a nitrocellulose membrane. FLCs were detected by specific antibodies and an enhanced chemiluminescence detection system. The FLC concentrations were calculated. We studied 15 healthy subjects, 10 patients with chronic renal failure, 71 patients undergoing haemodialysis treatment and 33 patients treated with haemodiafiltration. Different membranes were compared: low- and high-flux polysulfone membranes, low- and high-flux cellulose triacetate membranes, high-flux polymethylmethacrylate and polyacrylonitrile membranes.

Results. Chronic renal failure patients showed elevated FLC concentrations as compared with controls. In haemodialysis or haemodiafiltration patients these values were even higher. This was mainly due to an increased concentration of FLC of the λ-type. The treatment modality *per se* did not influence the FLC concentrations. Only haemodialysis or haemodiafiltration with the polymethylmethacrylate membrane lead to a significant reduction in FLC concentrations; however, these did not reach control levels. We did not observe differences in FLC levels between patients with different underlying diseases, nor did we find a correlation between age or the duration of the dialysis treatment and FLC concentrations. We found a positive correlation between FLC concentrations at the beginning of dialysis treatment and the amount of IgLCs removed during treatment. However, the average FLC level after treatment did not reach control values.

Conclusions. Currently available haemodialysis or haemodiafiltration treatments are unable to normalize the elevated serum/plasma levels of FLCs in end-stage renal disease patients.

Keywords: chronic renal failure; haemodialysis; immunoglobulin light chains; kappa chains; lambda chains

Introduction

Uraemic toxins are retention solutes that accumulate in the serum/plasma of patients with a reduced kidney function and contribute to a variety of metabolic and functional disturbances, such as a diminished immune defence. Recently, we have demonstrated that free immunoglobulin light chains (FLCs) are able to modulate functions of polymorphonuclear leukocytes (PMNL) and can be considered as members of the family of uraemic toxins [1]. Usually, there is a slight overproduction of FLCs as compared with heavy chains [2]. Therefore, an intracellular pool of FLCs (κ- and λ-type) exists within immunoglobulin-producing cells. FLCs are also found in human plasma, most likely as a result of a secretion parallel to the secretion of intact immunoglobulins, i.e. they originate by *de novo* synthesis and do not represent degradation products. FLCs accumulate in the sera of uraemic patients as a result of a reduced or abolished clearance by the kidney.

Wagasugi *et al.* [3] reported in a study published in 1991 that haemodialysis (HD) treatment was not able to lower FLC levels in serum/plasma and that patients receiving HD have even higher FLC serum/plasma levels than pre-HD patients with chronic renal failure (CRF). Since then, there has been no report on the removal of FLCs by HD. We have therefore investigated in the present study the effect of HD and haemodiafiltration (HDF) with different synthetic membranes to answer the question of whether currently available HD or HDF treatments can

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normalize FLC levels in serum/plasma in end-stage renal disease patients. We used a novel assay based on the immunodetection of FLCs separated from intact immunoglobulins by electrophoresis.

Subjects and methods

Patients

We studied 15 healthy subjects, 10 patients with chronic renal failure and 71 patients undergoing regular haemodialysis treatment. Eighteen of these patients were haemodialysed with high-flux polysulfone (F60S; Fresenius, Oberursel, Germany), nine patients with low-flux polysulfone (F6S; Fresenius), 14 patients with low-flux cellulose triacetate (N150; Nipro; Osaka, Japan), 17 patients with high-flux cellulose triacetate (N210; Nipro), four patients with polymethylmethacrylate (PMMA; Toray; Tokyo, Japan), and nine patients with polyacrylonitrile (AN69; Hospal; Meyzien Cedex, France). Furthermore, 33 patients underwent HDF treatment. Eighteen of them were treated with the F60 membrane, 11 with the N210 membrane, and four with the PMMA membrane. The overall K*t*/V (mean $\pm$ SEM) of the patients was 1.36 ± 0.02. No clinical signs for infection were found in any of the patients. Informed consent was obtained from all patients.

Determination of FLC concentrations in sera/plasma

Phosphate-buffered saline pH 7.4 (PBS; 30 µl) or 30 µl light chain solution of known concentration (internal standard) was added to 30 µl serum. For κ FLCs we used purified human Bence Jones kappa (P 016; Nordic Immunology, Tilburg, the Netherlands) and for λ FLCs purified human Bence Jones lambda (P 017; Nordic Immunology). The protein concentration of the added FLCs was determined by the bicinchoninic (BCA) protein assay (Pierce, Rockford, IL). To avoid interference in FLC dimer ($\sim$50 kDa) detection due to the presence of albumin ($\sim$68 kDa), albumin was largely removed from the serum by Blue–Sepharose treatment prior to analysis. Blue Sepharose 6 Fast Flow (Amersham Pharmacia Biotech AB, Uppsala; Sweden) was washed twice with 2 vol. PBS, 140 µl of the Blue–Sepharose suspension was added, and the samples were incubated at 37°C for 30 min on a shaker. The Blue–Sepharose was then spun down and 10 µl of non-reducing SDS–PAGE sample buffer was added to 10 µl supernatant. After 15–30 min incubation at 37°C the serum proteins were separated by non-reducing SDS–PAGE (12.5% homogenous gel, Phast System; Pharmacia LKB Biotechnology, Uppsala, Sweden) and electroblotted (Phast System) onto a nitro-cellulose membrane (Hybond ECL, Amersham Pharmacia Biotech, Buckinghamsire, UK). Four samples with and four samples without light chain addition were run on one gel. The quantity of added light chains was in the same range (up to twofold) as the FLCs in the serum. This approximate concentration was determined by running all sera on a non-reducing SDS–PAGE gel; a sample with known light chain concentration (100 µg/ml) was run on each gel as an external standard.

The transferred FLCs were detected with antibodies specific for κ and λ FLCs (K1255 and L7646, respectively; Sigma, St Louis, MO, USA), horseradish peroxidase (HRP)-labelled goat-anti-rabbit antibodies (NIP824; Amersham Pharmacia Biotech) and the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech) using Hyperfilm ECL (Amersham Pharmacia Biotech). The intensity of each of the κ and λ FLC bands in the monomeric and dimeric form was determined by scanning with the Ultro Scan XL (Pharmacia LKB) and the image master 1D program (Amersham Pharmacia Biotech). Using the known amount of added light chains, the concentration of FLCs in the serum was calculated.

Preparation of whole Igs

Serum from a healthy donor was passed through a 5-ml protein G column (Amersham Pharmacia Biotech). Whole Igs were eluted by lowering the pH (0.2 M Glycine–HCl, pH 2.8) and neutralized immediately after elution by a 1/5 vol. of 1 M Tris–HCl, pH 8.0. Igs were separated further from small amounts of co-eluting proteins of lower molecular weight by size-exclusion fast-protein liquid chromatography (FPLC, Superdex 200 prep grade, high load 16/50; Amersham Pharmacia Biotech).

Statistical analysis

All values are expressed as means $\pm$ SEM (standard error of the mean). The FLC levels were compared by analysis of variance (ANOVA). For paired samples we used the Wilcoxon test. P values <0.05 were considered statistically significant.

Results

Figure 1 shows the western blot of the separation of intact Igs, and FLC monomers and dimers of a uraemic serum by non-reducing SDS–PAGE (lanes b and e). In lanes a and d, a preparation of intact Ig without FLCs was loaded. We loaded 1000-fold more intact Ig onto the gel, because the Ig levels in human sera are $\sim$1000 times higher than the levels of FLC. No light chains were released under the experimental conditions used for this assay. The anti-κ antibody did not cross react with λ chains and the anti-λ antibody did not cross react with κ chains (data not shown).

Table 1 shows the results of a recovery assay. Different amounts of κ- or λ-light chains were added to a human control serum with known, i.e. previously measured, light chain concentrations. The dilutions of the samples were considered. The recovery values obtained with our assay were between 93 and 109%.

Table 2A shows the serum/plasma concentration of total FLCs and of κ- and λ-light chains in normal healthy adults, in CRF patients, and in dialysis patients treated by HD or HDF. In CRF patients, both κ- and λ-FLC concentration is higher than in controls. In the group of HD/HDF patients, the FLC concentrations are further increased. Whereas the concentrations of the κ chains are approximately twofold higher than controls, the level of λ chains is increased threefold. This is also reflected by the lower κ/λ ratio observed in the group of dialysis patients (HD/HDF patients: 0.80; healthy subjects: 1.10). CRF

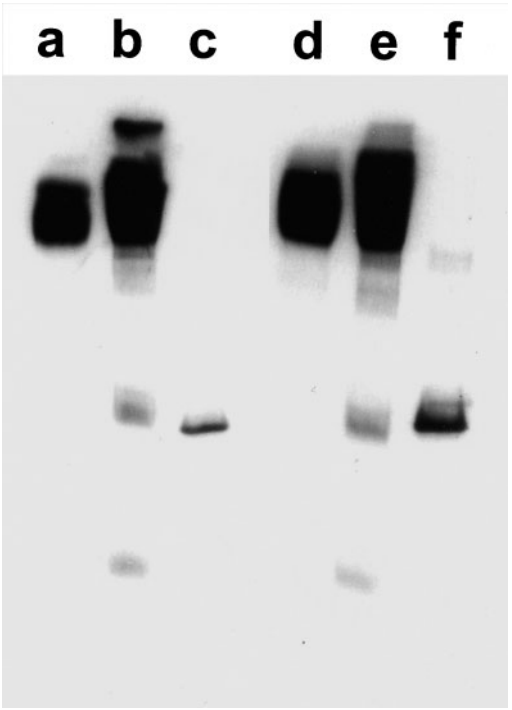

Fig. 1. Western blot of a nonreducing SDS-PAGE of isolated intact Ig (a, d) with a concentration of 18 mg/ml, of a uraemic serum/plasma treated with Blue Sepharose (b, e), and of commercially available κ-(c) and λ-(f) FLC with a concentration of 18 μg/ml. The blot was developed with anti-κ antibody (a–c) or with anti-λ antibody (d–f).

Table 1. Recovery assay: known amounts of κ- or λ-light chains were added to human control serum

Assay	Original concentration (μg/ml)	Amount added (μg/ml)	Expected (μg/ml)	Measured (μg/ml)	Recovery (μg/ml)
Kappa	36	10	46	44	96
		30	66	64	97
		100	136	133	98
Lambda	19	10	29	27	93
		30	49	46	94
		100	119	130	109

The measured data are the mean values of three separate experiments.

patients, however, have a higher κ/λ ratio than both healthy subjects and HD/HDF patients.

Table 2B compares the serum/plasma light chain concentrations in patients undergoing therapy with high- and low-flux dialysers, with those on HD/HDF with PMMA, before the start and after 2 h of HD or HDF. A significant light chain reduction was only obtained in patients undergoing HD or HDF with PMMA. This was mainly the result of a significant decrease in the concentration of λ-type light chains.

We found a positive correlation between the light chain concentration at the beginning of HD/HDF treatment and the amount of light chains removed during dialysis. Whereas we observed an increase

Table 2. Concentrations of κ- and λ-FLCs, their ratio, and the concentration of total FLCs in serum/plasma of healthy adults, patients with chronic renal failure, and patients undergoing haemodialysis or haemodiafiltration treatment (A), and in HD/HDF patients before and 2 h after the start of treatment with PMMA or other high- and low-flux membranes (B)

	κ (μg/ml)	λ (μg/ml)	LC (μg/ml)	κ/λ (μg/ml)	n
A					
Co	34 (4)	31 (3)	65 (6)	1.10	15
CRF	57 (11)	49 (13)	106 (22)	1.16	10
HD/HDF	70 (6)[a]	87 (6)[b]	157 (11)[b]	0.80	104
B					
LF pre	54 (7)	74 (11)	128 (16)	0.73	23
LF post	56 (8)	76 (9)	132 (14)	0.74	23
HF pre	77 (7)	88 (7)	165 (12)	0.88	73
HF post	76 (5)	93 (8)	169 (11)	0.82	73
PMMA pre	50 (8)	109 (21)	159 (26)	0.46	8
PMMA post	44 (12)	62 (10)[c]	106 (16)[d]	0.71	8

Values are represented as the mean (SEM). LC, total FLCs; Co, healthy adults; CRF, chronic renal failure; HD/HDF, haemodialysis or haemodiafiltration treatment; pre, pre-treatment; post, 2 h after the start of treatment; HF, high-flux membranes; LF, low-flux membranes.
HD/HDF *vs* Co: [a]$P<0.05$; [b]$P<0.01$.
PMMA post *vs* PMMA pre: [c]$P<0.025$; [d]$P=0.025$.

in light chain levels during HD/HDF treatment in patients with low pre-dialysis concentrations of light chains, the dialysis treatment lead to a significant reduction in light chain levels only in patients with very high pre-dialysis concentrations (Figure 2).

Finally, we analysed the FLC concentrations (total, κ and λ) in serum/plasma of HD/HDF patients according to their underlying diseases. There were no differences in FLC levels between these groups (data not shown). We did not find a correlation between FLC concentrations and age or between FLC concentrations and the duration of HD/HDF treatment (data not shown).

Discussion

The removal of uraemic retention solutes by renal replacement therapies is currently the best treatment modality for renal failure patients. FLCs have previously been shown to be uraemic toxins. In the present work we investigated the effect of HD and HDF on serum/plasma FLC levels in uraemic patients.

FLCs isolated from HD and continuous ambulatory peritoneal dialysis patients inhibit PMNL chemotaxis and the stimulation of glucose uptake [1]. On the other hand, FLCs stimulate basal levels of glucose uptake and of the oxidative metabolism of PMNL [4]. Furthermore, the presence of FLCs increases the percentage of viable PMNL by inhibiting spontaneous apoptotic cell death [5]. Therefore, FLCs may not only contribute to the diminished immune function but also to the state of baseline pre-activation of the immune system in uraemia.

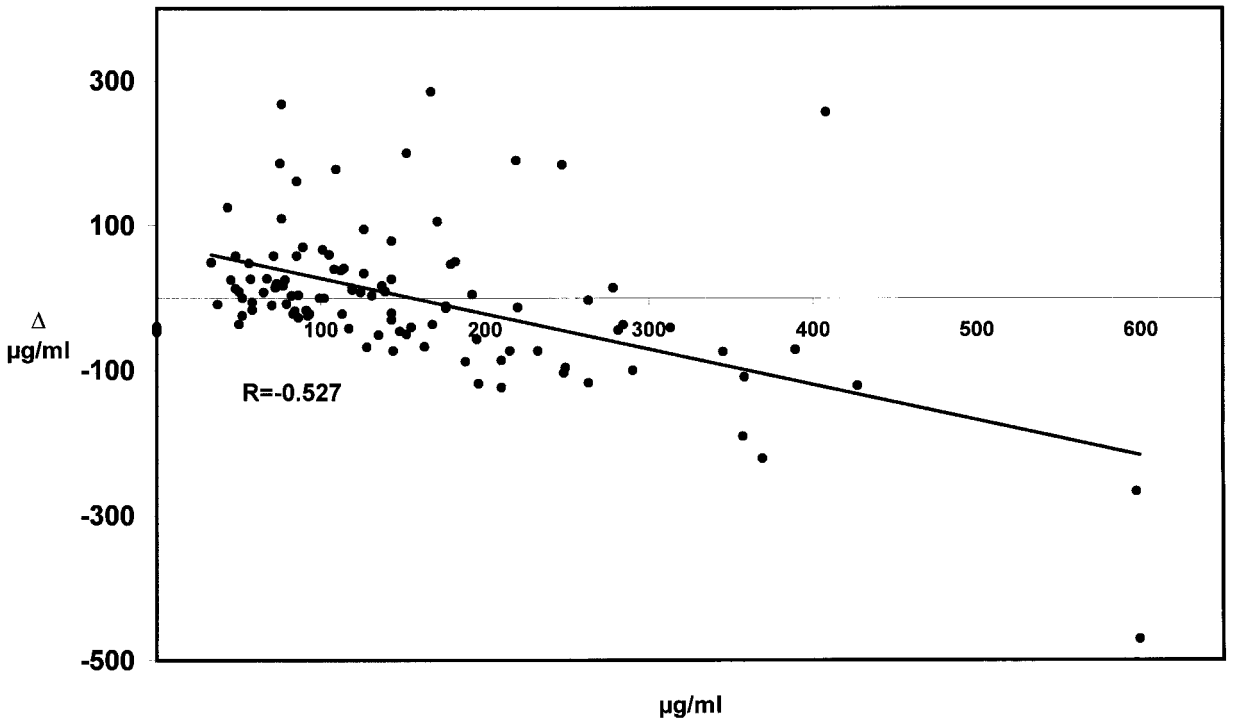

Fig. 2. Correlation between the concentrations of total FLCs in patients undergoing haemodialysis or haemodiafiltration treatment (HD/HDF; $n=104$) before the start of the treatment and the respective difference of the concentration 2 h after the start of HD or HDF. Mean value $\pm$ SEM; $^{*}P<0.05$.

Kappa- and λ-type FLCs exist as monomers and dimers with molecular weights of approximately 25 and 50 kDa, respectively. Significantly increased levels of FLCs in sera from patients with severely reduced kidney function has been reported by Solling [6] and by Wagasugi *et al.* [3]. Whereas there is a negative correlation between FLC serum concentration and glomerular filtration rate [6], there is no significant increase in the concentration of intact Igs in uraemia [3].

The focus of the present work is the measurement of FLC levels in serum/plasma of patients undergoing regular HD or HDF before and 2 h after the start of treatment. We used a method based on the separation of serum proteins by non-reducing SDS–PAGE. This allowed us to measure FLCs in their monomeric and dimeric forms independently from Igs, which exist in a 1000-fold excess in normal human serum. However, one must be aware that because FLCs have a tendency to polymerize that is dependent on the protein concentration [7], the measured monomer to dimer ratio does not necessarily reflect precisely the actual values in the sample. Other FLC tests use antibodies that react only with free IgLCs [6,8]. However, only a small subpopulation of Igs, denatured in a manner such that unexposed epitopes on the LCs are turned outward, would be enough to interfere with FLC determination. As shown in Figure 1, whole Igs stayed intact during our assay procedure and therefore did not interfere with the determination of FLC levels.

Table 2A shows that the FLC concentration in HD patients is even higher than in CRF patients. This is mainly due to the higher λ chain concentration. This was also found by Wakasugi *et al.* [3] who demonstrated a predominance of λ chains in HD patients. Despite some differences in the actual concentrations, partly due to the different pool of donors, the results regarding CRF patients published by Solling [6] correspond to ours in the following points: (i) in the sera of CRF patients, the FLC concentrations of both κ- and λ-type are higher than in the sera of healthy controls; and (ii) in CRF patients the κ/λ ratio is higher than in the controls. The κ/λ ratio of light chains as part of the intact Igs is 1.86 [9]. The lower ratio of FLCs in normal sera is explained by the faster renal elimination of free κ- compared with λ-light chains as a result of the difference in polymerization behaviour [10]. Impaired renal elimination, therefore, causes a relative increase in κ light chains.

The increase in FLC concentration in HD patients does not correlate with the duration of HD therapy, as demonstrated by our group (data not shown) and by Wakasugi *et al.* [3]. An increased FLC concentration was found in all groups of renal failure patients with different underlying diseases. We did not find any correlation between age and FLC concentration. Solling [6] measured FLC concentrations in different age groups and found a gradual increase from 1- to 10–20-year-old people. For adults, the FLC concentration did not change any more with increasing age.

As we investigated only sera from adults our results are in agreement with those of Solling.

Table 2B shows the effect of HD or HDF treatment with different membranes on FLC concentrations. Only treatment with the PMMA membrane significantly decreased FLC concentrations, especially of the λ-dimer, which has been found at very high levels in the serum/plasma of patients undergoing dialysis treatment. The HDF treatment with other membranes did not lead to a significant decrease in FLC levels. Therefore, we conclude that the removal of FLCs by the PMMA membrane is mainly due to adsorption. This is in agreement with other reports concerning the adsorptive properties of the PMMA membrane: $>85\%$ of factor D [11] and $>90\%$ of β2-microglobulin removal [12] occurs by adsorption using PMMA. On the other hand, we did not find a significant FLC reduction using the polyacrylonitrile membrane, a dialyser also known for high protein adsorption.

The average FLC concentration 2 h after the start of treatment did not reach control levels. Furthermore, we found that the amount of FLCs removed by dialysis treatment decreases with decreasing serum/plasma FLC levels and that dialysis treatment is most efficient for very high FLC concentrations (Figure 2). A similar situation has been reported for β2-microglobulin [13].

Our observation suggests the existence of two different counteracting mechanisms influencing light chain levels during HD/HDF. The dialysis treatment *per se* seems to lead to an increased rate of light chain appearance in the serum/plasma by a yet unknown mechanism. This effect is most pronounced at low light chain concentrations. Only at very high light chain levels does the second mechanism, light chain removal by dialysis and/or adsorption, become dominant and lead to concentrations which are still far higher than normal.

Therefore, we conclude from our results that currently available HD or HDF treatments cannot normalize elevated serum/plasma levels of FLCs in end-stage renal disease patients.

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