Effects of a polyelectrolyte additive on the selective dialysis membrane permeability for low-molecular-weight proteins

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Abstract
Background. Improving the sieving characteristics of dialysis membranes enhances the clearance of low-molecular-weight (LMW) proteins and may have an impact on outcome in patients receiving haemodialysis. To approach this goal, a novel polyelectrolyte additive process was applied to a polyethersulphone (PES) membrane.

Methods. Polyelectrolyte-modified PES was characterized in vitro by measuring complement activation and sieving coefficients of cytochrome c and serum albumin. In a prospective, randomized, cross-over study, instantaneous plasma water clearances and reduction rates of LMW proteins [beta2-microglobulin (b2m), cystatin c, myoglobin, retinol binding protein] were determined in eight patients receiving dialysis treatment with PES in comparison with polysulphone (PSU). Biocompatibility was assessed by determination of transient leucopenia, plasma levels of complement C5a, thrombin-antithrombin III (TAT), myeloperoxidase (MPO) and elastase (ELT).

Results. PES showed a steeper sieving profile and lower complement activation in vitro compared with PSU. Instantaneous clearance (69 ± 8 vs. 58 ± 3 ml/min; \(P < 0.001\)) and reduction rate (72.3 ± 1.5% vs. 66.2 ± 6.1%; \(P < 0.001\)) of b2m were significantly higher with PES as compared with PSU. With higher molecular weight of proteins, differences in the solute removal between PES and PSU further increased, whereas albumin loss remained low (PES, 0.53 ± 0.17 vs. PSU, <0.22 g/dialysis). MPO, ELT and TAT did not differ between the two membranes. In contrast, leucopenia was less pronounced and C5a generation was significantly lower during dialysis with PES.

Conclusions. Polyelectrolyte modification of PES results in a higher LMW protein removal and in optimized biocompatibility. Whether these findings translate into better outcome of patients receiving haemodialysis requires further studies.

Keywords: biocompatibility; dialysis membrane; end-stage renal disease; haemodialysis; low-molecular-weight-protein removal; polyelectrolyte additive

Introduction
To date, about 90 different uraemic toxins have been identified. These substances represent only a minority of solutes which accumulate during chronic renal failure and contribute to the uraemic syndrome [1]. Uraemic toxins comprise a wide range of molecular weights (MW), but only low-molecular-weight (LMW) proteins smaller than albumin (MW ~ 67 000 Da) represent a target for direct elimination by non-selective extracorporeal renal replacement therapies. The loss of larger molecules may deplete essential proteins and generate hypoalbuminaemia with the potential consequence of a worsened patient outcome [2].

The elimination of LMW proteins much larger than urea must be beneficial for end-stage renal disease (ESRD) patients. This conclusion is older than 30 years and led to the development of high-flux dialysis membranes [3]. Beta2-microglobulin (b2m), a prototype middle molecule of 11 800 Da, has become the most important surrogate parameter to measure dialysis efficiency regarding middle molecule removal in vivo. Convective therapies with enhanced b2m removal seem to improve outcome not only with respect to dialysis-related amyloidosis, in which b2m accumulation plays a major role [4–7]. First reports indicate that ESRD patients with lower b2m plasma levels are at lower relative risk for mortality [8,9].
However, it is clear that b2m represents only one accumulating larger LMW protein in uraemia.

A current trend in dialysis membrane engineering is to maximize the permeability for larger LMW proteins while retaining albumin. Protein-leaking dialysis membranes do not meet these requirements. In convective procedures, such as haemodiafiltration, their albumin leakage is too high [10]. Application of nanotechnology production principles has been claimed to represent a way to modulate and control pore morphology of a synthetic membrane [11]. With these techniques, significantly increased sieving coefficients for molecules, such as b2m, while maintaining an extremely low albumin loss, were achieved [12].

PUREMA® H is a new polyethersulphone (PES) dialysis membrane, produced by applying an innovative spinning technique. The incentive of its development was to improve the characteristics of existing dialysis membranes, i.e. realizing a steeper sieving profile for LMW proteins at best biocompatibility properties, for a more adequate dialysis therapy.

This study presents the first in vitro and in vivo data to introduce a novel polyelectrolyte additive process to dialysis membrane technology.

**Methods**

**In vitro characterization**

The new PES is a synthetic dialysis membrane with an asymmetric wall structure composed of a PES-polyvinylpyrrolidone blend. By specific addition of negatively charged polyelectrolytes to the inner separating layer of the membrane during the spinning process, a modification of the ζ-potential of this blood-contacting layer has been obtained. To provide information about electrosurface phenomena related to structural features of the polymer surfaces, the isoelectric point (IEP) and the ζ-potential of PES were quantified in comparison with polysulphone (PSU: Helixon®, Fresenius Medical Care AG, Bad Homburg, Germany) and the AN69 was measured at 5, 10, 15, 20, 30 and 60 min of dialysis in blood, as described previously [16].

**In vitro sieving coefficients (S)** for cytochrome c (12 400 Da) and albumin (~67 000 Da) were determined in PES and PSU minidialysers (surface area 0.02 m²), the latter serving as a control. Cytochrome c (100 mg) and bovine serum albumin (50 g) added to 1.000 ml phosphate-buffered saline (PBS) solution was pumped single-pass over 50 min at a flow rate of 200 ml/min/m² through the minidialysis blood compartment. The ultrafiltration rate (QF) was set at a flow rate of 200 ml/min/m² through the minimodule saline (PBS) solution was pumped single-pass over 50 min serum albumin (50 g) added to 1.000 ml phosphate-buffered solution serving as a control. Cytochrome c (100 mg) and bovine albumin (1.000 ml) was recirculated at 250 ml/min for 180 min at 37°C. After baseline, blood samples for complement receptor CR3 and C5a determination were drawn at 60 and 180 min, respectively, using a flow cytometer for CR3 (FACScan, Becton-Dickinson, Heidelberg, Germany) and an ELISA for C5a (C5a Micro ELISA, DRG Instruments GmbH, Marburg, Germany).

**Animal study design**

After having received approval from the district government of Lower Franconia (registration no. 621-2531.01-38/03), haemodialysis was performed in four sheep to test for anaphylactoid reactions and the generation of bradykinin in blood, as described previously [16]. After pre-treatment with a daily oral dose of 30 mg enalapril for four days prior to the experiments, the sheep were haemodialysed for 1 h in a cross-over fashion with the PES (PUREMA® H, 1.7 m², Membrana GmbH, Wuppertal, Germany) and the AN69® (Filtral®, 1.6 m², Hospal, France) dialysis membrane. AN69® was used to serve as a positive control and was taken from retained samples since its distribution was stopped several years ago. During dialysis, the animals were observed for symptoms of anaphylactoid reactions and blood pressure was measured via tail cuff by using an automated device [16]. Angiotensin-converting enzyme and bradykinin levels in blood were determined at baseline. Bradykinin was also measured at 5, 10, 15, 20, 30 and 60 min of dialysis in blood leaving the dialysers. Blood samples were processed as described previously [16].

**Clinical study design**

Study approval was given by the Freiburg Ethics Committee International (registration no. 04/1077). The study design was prospective, randomized and cross-over.

Eight stable ESRD patients (mean age 62.1 ± 13.8 years; five female, three male) on regular thrice-weekly haemodialysis treatment were enrolled into the study after they had given written informed consent. Underlying renal diseases were glomerulonephritis (n = 3), diabetic nephropathy (n = 2), autosomal dominant polycystic kidney disease (n = 2) and interstitial nephritis (n = 1). The mean duration on dialysis treatment was 76.0 ± 55.3 months.
(range 20–195 months). The mean post-dialysis body weight was 68.5 ± 7.5 kg. All patients were anuric and had well-functioning native AV fistulas for blood access. The patient’s concomitant medications were continued in an unchanged manner including anticoagulation under study conditions. Each patient underwent randomly one study week of three consecutive haemodialysis treatments with the PES dialysis membrane (PUREMA® H, 1.7 m², Membrana GmbH, Wuppertal, Germany) built in a standard dialyser housing and one week with the PSU control high-flux dialysis membrane (Helixone®, Fresenius FX80 dialyzer, 1.8 m², Fresenius Medical Care AG, Bad Homburg, Germany).

Haemodialysis was performed using Gambio AK 200 S monitors (Gambio,Lund, Sweden). The treatment duration was kept constant for each individual patient at 236 ± 11 min for both test and control membranes.

Blood flow (Qb) and dialysate flow (Qd) rates were set constant at 300 and 500 ml/min, respectively.

QUF of each session was set according to individual patient’s inter-dialytic weight gain and was not different for both membranes (PES 2900 ± 816 ml; PSU 2838 ± 746 ml). Anticoagulation was performed by unchanged adoption of form and dosage of the previous routine heparinization. Six patients received standard heparin and two received fractionated heparin.

Quantitation of dialysis membrane efficacy

Treatment efficacy was determined during the third (mid-week) consecutive session with each respective membrane by measuring instantaneous whole blood clearances (K) and reduction ratios (RRs). The total mass of transmembrane albumin loss (MTD) was detected in continuously collected dialysate.

K was measured after 30 min and at the end of the treatment for the small solutes urea (60 Da), creatinine (113 Da), and phosphate (96 Da) and for larger substances b2m, cystatin c (cysc; 13 400 Da), myoglobin (myo; 17 800 Da), and free retinol-binding protein (rbp; 21 200 Da). Plasma concentrations were determined in blood samples obtained from the arterial (Cart) and the venous (Cven) blood line of the extracorporeal circuit. During sampling, QUF was maintained. QUF was taken into account for calculation of K (Equation 4) [17]:

\[ K_{\text{solute}} = Q_B \times \left( \frac{C_{\text{art}} - C_{\text{ven}}}{C_{\text{art}}} \right) + \left( Q_{\text{UF}} \times \frac{C_{\text{ven}}}{C_{\text{art}}} \right) \text{ (ml/min)} \] (4)

Since whole blood clearances overestimate real values, plasma water clearances were calculated according to Equation (5) using the patient’s haematocrit level (Hct) at the time of the respective clearance sampling and the predialysis total protein level (TP, g/l) [14]. To account for solute shift from the blood cell, solute partition coefficients (SPC) were assumed as 0.86 (urea), 0.73 (creatinine), 0.5 (phosphate), and 0 (b2m, cysc, myo and rbp) [14].

\[ K_{\text{plasma}} = K_{\text{solute}} \times \left( 1 - 0.0107TP \right) \times SPC \times Hct \] (5) + (1 - Hct)

RR was determined for the LMW proteins b2m, cysc, myo and rbp. Plasma concentrations were measured in blood samples drawn from the arterial blood line before (Cpre) and at the end (Cpost) of each treatment after reduction of Qb to 50 ml/min and dialysate flow turned off for 30s. RR was calculated according to Equation (6) using corrected Cpost (Cpost-corr) [18]. Cpost was corrected for extracapillary volume changes based on differences in the patient’s pre- (BWpre) and post-dialysis body weight (BWpost) (Equation 7) [19].

\[ RR = 1 - \frac{C_{\text{post-corr}}}{C_{\text{pre}}} \times 100 \% \] (6)

\[ C_{\text{post-corr}} = \frac{C_{\text{post}}}{1 + (\text{BWpre} - \text{BWpost})/0.2 \times \text{BWpost}} \] (7)

The mass transfer into dialysate (MTD) was determined by continuously collecting a spent dialysate fraction of 10 ml/min during the whole haemodialysis duration (t) via a T-connector inserted into the dialysate drainage line. The albumin concentration (CAlb) was determined in an aliquot taken from the collected spent dialysate. The albumin mass in dialysate was calculated according to Equation (8).

\[ M_{\text{TD}} = C_{\text{Alb}} \times (Q_D + Q_{\text{UF}}) \times t \text{ (mg)} \] (8)

All LMW protein concentrations were measured by laser immunonephelometry (BN 100 Analyzer, Dade Behring GmbH, Marburg, Germany).

Assessment of dialysis membrane biocompatibility

Biocompatibility was assessed by measuring white blood count (WBC), complement factor C5a, thrombin-antithrombin III-complex (TAT), myeloperoxidase (MPO) and elastase (ELT) generation over time.

Baseline values were determined from blood drawn from the patients’ arteriovenous fistula. After initiation of dialysis, all samples were taken from the venous line of the extracorporeal circuit except for WBC which was derived from the arterial line.

WBC and C5a were determined before, at 5, 10, 15, 30 and 60 min and at the end of dialysis. TAT, MPO and ELT were measured before, at 30 and 60 min and at the end of treatment.

For WBC determination, an automatic cell counter was used. C5a, TAT (Enzygnost TAT Micro, Dade Behring GmbH, Marburg, Germany), MPO (MPO ELISA, Immundiagnostik, Benzheim, Germany) and ELT (Milenia PMN-elastase, Milenia Biotech GmbH, Bad Nauheim, Germany) plasma concentrations were measured by applying commercially available ELISA kits.

Data analysis

Descriptive analysis of the results was performed by calculating mean values ± standard deviations (SD). In vitro results were analysed by ANOVA and a Tukey post hoc test. Comparative statistical analyses of within-subject within-treatment changes from baseline and within-subject between-treatment differences were assessed using the two-sided paired t-test. A P-value of <0.05 was considered as statistically significant. Statistical evaluation was performed by means of the ‘Statgraphics 5.0 plus’ software package (Manugistics, Inc., Rockville, USA).
Results

In vitro characterization

For both PES with and without polyelectrolyte additive, the IEP of the membrane surfaces was 3.4. It was slightly shifted to the acid side in comparison with PSU (IEP value of 3.9). IEPs below pH 4 indicate the presence of acidic surface sites. At neutral pH 7.4, the ζ-potential of PES with additive was −52 mV, while for PES without polyelectrolyte additive and for PSU the absolute value of the ζ-potential was lower, being −45 and −31 mV, respectively. Compared with PSU, PES displayed a steeper sieving profile for LMW proteins, as measured by cytochrome c and albumin sieving coefficients, and a higher ultrafiltration coefficient (Table 1). Furthermore, PES led to significantly lower complement C5a generation and CR3 up-regulation (Table 1).

Animal experiments

Haemodialysis with the AN69® membrane led to anaphylactoid reactions in the form of tachypnoe, hypersalivation and hypotension in all sheep. After 5 min of treatment, the systolic blood pressure decreased from initially 110±3.6 mmHg to below 50 mmHg, the lower sensitivity limit of blood pressure determination by tail cuff. Collapse occurred simultaneously in two of the four animals. All sheep recovered from the anaphylactoid symptoms within a few minutes after onset. In contrast, the experiments with PES were completely uneventful. The systolic blood pressure slowly decreased over time without sudden alterations during the early minutes of dialysis.

Angiotensin-converting enzyme activity before enalapril was 42.9±0.16 U/ml. After pre-treatment with enalapril, the baseline levels were reduced (P < 0.05) to 0.16±0.21 U/ml in AN69® and 3.36±0.71 U/ml in PES.

Bradykinin levels in AN69®-treated sheep increased significantly (P < 0.05) from 14.2±0.9 to a maximum of 7006±2064 pg/ml after 5 min and did not return to baseline concentrations at the end of dialysis. Throughout the whole dialysis treatment, no changes of the bradykinin concentrations were measured in PES (63.6±14.7 pg/ml at baseline and 33.5±8.4 to 59.0±29.1 pg/ml between 5 and 60 min).

Clinical trial

Treatment efficiency. Small solute instantaneous clearances were similar for both PES and PSU (Table 2). Compared with 30 min, significantly lower values were found almost always at the end of the treatment. Clearances for b2m, cysteine and myo were substantially higher in PES compared with PSU independently of time. For b2m, this difference was 19.0% and 26.0% at 30 min and at the end of the treatment, respectively. The differences in cysteine clearances amounted to 52.3% and 58.3% and in myo clearances to 233.3% and 162.5% at 30 min and at the end of the treatment, respectively (Table 2). The significant clearance differences (P < 0.01) favouring PES increased with higher MW (Figure 1). For rbp, negative clearance values were determined for both dialysis membranes.

The b2m RR with PES was 9.2% higher (72.3±5% vs 66.2±6.1%) and the plasma removal of cysteine and myo was enhanced by 25% (69.5±4.6% vs 55.6±10.1%) and 124.8% (51.7±7.3% vs 23±10.4%), respectively (Figure 2).

A significant (P < 0.01) higher albumin loss of 0.53±0.17 g was found for PES. For PSU, the albumin mass transfer remained throughout below 0.22 g, the lower detection limit of the albumin assay.

Biocompatibility. Lowest WBC for PSU was determined after 10 min of dialysis being 81.5±7.8% compared with baseline, but a significant decrease was observed already after 5 min. The WBC nadir for PES (91.6±13.4%; P = non-significant) was measured at 5 min (Figure 3). Leucocyte counts recovered with both dialysis membranes within 30 min without further major alterations. No statistically significant differences were found between the two dialysis membranes.

Peak C5a generation during dialysis with PSU was measured at 5 min being significantly different from baseline (0.4±0.5–2.9±2.3 mg/l; P < 0.05) and PES (Figure 4). Maximum C5a generation with PES was observed at 10 min (0.4±0.2–1.2±1.2 mg/l), but significant differences vs 0 min were not established before 30 min (P < 0.05). At the end of dialysis, C5a concentrations did not reach baseline levels with both membranes (Figure 4).

The course of TAT concentrations was almost identical with PES and PSU, which is best described as slowly increasing values from the start until the treatment end (PES 2.1±0.3–5.9±7.8 μg/l; P = 0.218; PSU 2.3±0.4–6.6±6.3 μg/l; P = 0.105) (no further data displayed).

MPO and ELT behaved in a rather different way. Baseline MPO levels were significantly different between PES and PSU (90.5±30.0 vs 54.7±20.3 μg/l, P < 0.01). Therefore, the MPO course is described relative to baseline values (Figure 5). A steep and significant (P < 0.001) MPO increase was noted after 5 min with peak concentrations at 10 min (PES 498±260% and PSU 577±244% relative to 0 min). After 60 min, MPO decreased slowly until the end of dialysis without returning to baseline values, not showing significant differences between the dialysis membranes.

ELT concentrations slowly accumulated until the end of dialysis (PES 42.8±25.2–93.3±25.7 μg/l, P < 0.001; PSU 52.2±23.2–107.8±48.3 μg/l, P < 0.01) after a slightly more pronounced increase during the first 15 min of treatment, which was steeper and significant (P < 0.05) for PES after 5 min (Figure 6). However, the course of ELT was almost identical for both PES and PSU.
ESRD patients with lower b2m plasma levels seem to be at a lower relative mortality risk [8,9]. One option to considerably reduce pre-dialysis b2m levels may be the prescription of high-flux dialysis. Thus, with respect to LMW protein removal, engineering more efficient high-flux dialysis membranes is a logical consequence.

Table 1. In vitro membrane characteristics of PES and PSU

<table>
<thead>
<tr>
<th>Membrane material</th>
<th>PES – polyelectrolyte</th>
<th>PES – polyelectrolyte</th>
<th>PSU – polyelectrolyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With</td>
<td>without</td>
<td></td>
</tr>
<tr>
<td></td>
<td>polyelectrolytes</td>
<td>polyelectrolytes</td>
<td>polyelectrolytes</td>
</tr>
<tr>
<td>Wall structure</td>
<td>Asymmetric</td>
<td>Asymmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>Fibre diameter (µm)</td>
<td>200</td>
<td>200</td>
<td>185</td>
</tr>
<tr>
<td>Wall diameter (µm)</td>
<td>30</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Sieving coefficient</td>
<td></td>
<td></td>
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<tr>
<td>Cytochrome c</td>
<td>0.95 ± 0.04</td>
<td>0.60 ± 0.05</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.001 ± 0.001</td>
<td>0.005 ± 0.002</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>Ultrafiltration coefficient (ml/h mmHg)</td>
<td>85.3 ± 11.7c</td>
<td>82.6 ± 11.5c</td>
<td>62.2 ± 12.5c</td>
</tr>
<tr>
<td>Complement C5α (ng/ml) At 180 min</td>
<td>4.6 ± 2.3</td>
<td>–</td>
<td>7.3 ± 0.7a</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.4 ± 0.1</td>
<td>–</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Complement CR3 (FU) At 60 min</td>
<td>165 ± 46.2c</td>
<td>–</td>
<td>209 ± 3.6</td>
</tr>
<tr>
<td>Baseline</td>
<td>116 ± 17.3</td>
<td>–</td>
<td>116 ± 17.3</td>
</tr>
</tbody>
</table>

*P < 0.001 vs PES with polyelectrolytes. **P < 0.001 vs PES without polyelectrolytes; †P < 0.01 vs PSU.

Table 2. Instantaneous LMW protein plasma water clearances of PES and PSU

<table>
<thead>
<tr>
<th>Instantaneous LMW protein plasma water clearances (ml/min)</th>
<th>Urea</th>
<th>Creatinine</th>
<th>PO₄</th>
<th>b2m</th>
<th>cysc</th>
<th>myo</th>
<th>rbp</th>
</tr>
</thead>
<tbody>
<tr>
<td>PES</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>30 min</td>
<td>225 ± 8</td>
<td>209 ± 8</td>
<td>195 ± 7</td>
<td>69 ± 8a</td>
<td>67 ± 6b</td>
<td>30 ± 9a</td>
<td>−6 ± 7</td>
</tr>
<tr>
<td>Treatment end</td>
<td>217 ± 6</td>
<td>197 ± 7c</td>
<td>189 ± 6c</td>
<td>63 ± 5b,c</td>
<td>57 ± 3b,c</td>
<td>21 ± 5b,d</td>
<td>−6 ± 5</td>
</tr>
<tr>
<td>PSU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>226 ± 5</td>
<td>209 ± 7</td>
<td>197 ± 4</td>
<td>58 ± 3</td>
<td>44 ± 3</td>
<td>9 ± 7</td>
<td>−13 ± 9</td>
</tr>
<tr>
<td>Treatment end</td>
<td>217 ± 5c</td>
<td>197 ± 8c</td>
<td>190 ± 5c</td>
<td>50 ± 5c</td>
<td>36 ± 5c</td>
<td>8 ± 6</td>
<td>−4 ± 6</td>
</tr>
</tbody>
</table>

Blood flow and dialysate flow rates were set at 300 and 500 ml/min, respectively. B2m, beta-2-microglobulin; cysc, cystatin c; myo, myoglobin; rbp, retinol-binding protein.

*P < 0.01 vs PSU; **P < 0.001 vs PSU; †P < 0.01 vs 30 min; ‡P < 0.05 vs 30 min; §P < 0.001 vs 30 min.

Discussion

ESRD patients with lower b2m plasma levels seem to be at a lower relative mortality risk [8,9]. One option to considerably reduce pre-dialysis b2m levels may be the prescription of high-flux dialysis. Thus, with respect to LMW protein removal, engineering more efficient high-flux dialysis membranes is a logical consequence.
Since the loss of significant amounts of albumin may be associated with disadvantages for the patient outcome [2], minimal albumin permeability at maximum sieving properties for LMW proteins smaller than albumin represents one membrane development requirement. Another one is an optimized biocompatibility, which may also positively impact on patient survival [20].

Both in vitro and in vivo study results demonstrate that the modified PES high-flux dialysis membrane fulfils the criteria mentioned above. Instantaneous LMW protein clearances and reduction rates are significantly superior compared with PSU and the albumin loss of about 0.5 g is by far within accepted limits [2].

PSU is regarded as one of the most efficient dialysis membranes suited for haemodialysis and convective therapies, such as haemodiafiltration. It is claimed that its excellent performance characteristics are achieved by applying nanotechnological principles during fabrication [11,12]. Polyethersulphone-based PES is made of a similar synthetic polymer. Different to PSU, it obtains a higher selective permeability for LMW proteins by a patented additive process using negatively charged polyelectrolytes during spinning.

Fig. 2. Reduction rate comparison of PES (gray bars) and PSU (black bars). Mean values±SD are given. Plasma concentrations are corrected for changes in extracellular volume due to ultrafiltration. b2m, beta2-microglobulin; cysc, cystatin c; myo, myoglobin; rbp, retinol-binding protein. *P < 0.001 and **P < 0.01 vs PSU.

Fig. 3. Course of WBC during haemodialysis. Mean values±SD relative to baseline values of PES (squares/dotted line) and PSU (diamonds/solid line) are given. *P < 0.05 and #P < 0.01 vs 0 min.

Fig. 4. Course of ELT concentrations during haemodialysis. Comparison of PES (squares/dotted line) with PSU (diamonds/solid line). The figure depicts mean values±SD relative to baseline values. *P < 0.05, #P < 0.01 and ##P < 0.001 vs 0 min, **P < 0.05 vs PES.

Fig. 5. Course of MPO concentrations during haemodialysis. Mean values±SD relative to baseline values of PES (squares/dotted line) and PSU (diamonds/solid line) are given. *P < 0.001 and #P < 0.01 vs 0 min.
The resulting chemical modification of the membrane is characterized by a higher absolute value of the \( \zeta \)-potential compared with PSU and also with PES without polyelectrolytes. Identical IEPs for both PES with and without additive indicate that only the absolute value of the \( \zeta \)-potential but not the acidity of functional groups of the PES membrane are modified by the polyelectrolytes. This means that the chemical composition of the membrane is not changed. More likely, only a rearrangement of polar polyvinylpyrrolidone molecules during membrane precipitation has been actuated by the polyelectrolytes. This altered configuration may be the key for enhanced LMW protein clearances. Compared with the untreated membrane, the polyelectrolyte modification translates into a higher cytchrome c, a solute of similar MW as b2m, and a lower albumin sieving coefficient \textit{in vitro}. Our bench results revealed a much steeper sieving profile of PES also in comparison with PSU, which was confirmed in the subsequently performed clinical \textit{in vivo} study. Small solute clearances were almost identical for both membranes with a trend to lower values at the end of the treatment. This is possibly a tribute to a decreased diffusion gradient, the driving force for transmembrane elimination of such substances [15], rather than membrane fouling. With rising MW of the LMW proteins, the clearance differences favouring PES increased significantly being >19% higher for b2m, >52% higher for cysc and >162% higher for myo. Despite identical sieving coefficients for albumin, the \textit{in vivo} albumin loss per dialysis session was also significantly higher in PES as compared with PSU, indicating the limitations of \textit{in vitro} experiments in predicting this kind of clinical results.

The \( RR \) reflected the differences in LMW protein clearances. For b2m, haemodialysis with PES averaged at a remarkably high value of 72.3 \( \pm \) 5.0%, which was significantly better than with PSU (66.2 \( \pm \) 6.1%). Comparisons with other studies on dialyser performance are difficult because of the often considerably differing treatment conditions. However, b2m \( RR \) of this order of magnitude have previously rather been seen in more convective therapy forms than in haemodialysis. In on-line post-dilution haemodiafiltration, the most efficient routine dialysis procedure, b2m \( RR \) of 72.7% have been demonstrated [21]. At identical treatment duration (4 h) and blood and dialysate flow rates (300 and 500 ml/min, respectively), this matches exactly the result achieved with PES in simple dialysis mode. In contrast, during post-dilution haemodiafiltration, in which a conventional high-flux PSU dialyser (Fresenius F80, 1.8 m\(^2\)) was used, 100 ml/min of substitution fluid were infused [21]. On-line post-dilution haemodiafiltration permits to fully exploit the sieving characteristics of high-flux membranes. Therefore, it would be also interesting to study the performance of PES in this more convective therapy form.

The differences in \( RR \) of LMW proteins rose significantly with increasing MW, illustrating the steeper sieving profile of PES compared with PSU. For cysc, the difference amounted to 25% and for myo it was about 125%. This more efficient removal of larger LMW proteins by PES opens the possibility to target a wider range of uraemic toxins similar to what is claimed for haemodiafiltration [22]. However, the capacity to remove toxins much larger than 20000 Da still seems to be limited in dialysis mode with both PES and PSU. For rbp, the surrogate LMW protein in this range, clearances averaged at negative values and the \( RR \) were negligibly low, indicating a clinically negligible removal of this solute. The reason for negative rbp clearances remains unclear. The clearance calculation accounted for ultrafiltration of plasma water to achieve the patient’s weight loss, which leads to haemoconcentration and an increase of LMW protein concentration in blood leaving the dialyser.

Modern high-flux dialysis membranes allow diffusion of larger LMW proteins, such as b2m [23]. Therefore, in addition to internal filtration, diffusion may considerably contribute to b2m clearance in haemodialysis. Compared with PSU, the thinner wall thickness of PES (30 vs 35 \( \mu m \)) represents a shorter diffusive distance [15]. Whether diffusive differences between PES and PSU may have contributed to the differences in larger LMW protein clearances cannot be excluded, but this issue has not been addressed in this study.

Retrospective analyses have shown that haemodialysis with synthetic dialysis membranes is associated with improved patient survival in ESRD [20]. This observation was mainly attributed to membrane biocompatibility. Synthetic membranes are generally regarded as to be highly biocompatible, since they lead to low complement activation and leucopenia, two classical parameters to characterizing biocompatibility in dialysis [20]. However, several other systems become altered during blood-membrane interaction. Among them are the coagulation system and imbalances of the oxidative and anti-oxidative system [24,25].

Anticoagulation in form of systemic heparinization was adopted unchanged from routine dialysis treatment for individual patients. Pro-coagulatory activity during the treatments was assessed by measuring TAT concentrations over time. No differences between PES and PSU were detected. TAT levels slowly increased until the end of dialysis to rather low values with respect to literature [24]. Despite the fact that more convection, which might drive the superior LMW protein removal in PES due to higher internal filtration, is associated with enhanced TAT generation [24], definite conclusions on pro-coagulatory activity cannot be drawn. That would have required a different study design with a focus on anticoagulation.

MPO originates from neutrophils activated during dialysis. MPO kinetics is useful for the characterization of dialysis membrane differences as a parameter for oxidative stress, a promoter of morbidity and mortality in ESRD patients [25]. Our results confirm a pronounced MPO generation up to 60 min of dialysis for both PES and PSU. Synthetic membranes are known
to generate less MPO than cellulosic dialysis membranes [25,26]. The two synthetic membranes tested in this study did not show significant differences. However, MPO values were significantly different at baseline and a carry-over effect cannot be excluded.

Plasma ELT generation during dialysis is the result of neutrophil degranulation after cell activation by the dialysis membrane. In contrast to MPO, the increase of ELT levels is more linear with a maximum at the treatment end. Dialysis using synthetic membranes is associated with lower ELT generation than with cellulosic membranes [26]. No differences in ELT concentrations were found between PES and PSU while kinetics was consistent with respect to other studies [26].

The causative role of complement activation in transient leucopenia during haemodialysis has been confirmed in numerous studies [27]. Both are well-established parameters for the characterization of dialysis membrane bioincompatibility. In contrast to cellulosic membranes, synthetic dialysis membranes generally activate complement only marginally and lead to less-pronounced leucopenia [27]. Accordingly, the increase of complement C5a within the first 10 min of dialysis with PES and PSU was rather low. However, C5a levels induced by PSU with a maximum at 5 min were significantly different from baseline and about 2.5-fold higher compared with PES. Consistently, the nadir of transient leucopenia, which is correlated with complement activation [27], was less pronounced in dialysis with PES than with PSU, although this difference was not statistically significant. It must be considered that C5a is a middle molecule of 11 000 Da. Compared with PSU, the LMW protein removal of PES in our study has proven to be significantly superior. Thus, enhanced removal of complement fragments rather than chemical properties of the membranes may explain the observed differences. Therefore, the hypothesis that the improved LMW protein elimination of PES may result in an improved biocompatibility with respect to complement and leucocyte activation seems to be plausible.

This assumption may also account for the unusual early nadir of transient leucopenia, which was at 10 min in PSU and at 5 min in PES. Other authors have observed lowest leucocyte counts at 15 min [25]. Among the intense removal of cell-activating substances and the influence of the dialysis membrane material, also simple differences in the timing of blood sampling, being the first sample 15 min after baseline, and inter-individual differences in cell reactivity between the patients may be considered to explain this observation.

PES has passed all relevant biological safety tests according to the ISO standard 10993. However, negatively charged dialysis membranes have shown to induce potentially life-threatening contact activation and bradykinin generation during haemodialysis [16]. Therefore, PES was tested for anaphylactoid reactions and bradykinin generation in an established haemodialysis animal model using sheep [16]. In this model, the dialysis membrane AN69®, which carries negatively charged sulphonate groups, served as a control. In contrast to AN69®, which was associated with severe anaphylactoid reactions and an intense early bradykinin release, haemodialysis with PES was completely uneventful and plasma bradykinin concentrations remained unchanged in angiotensin-converting-enzyme-inhibited sheep. Apart from the harmlessness of PES, this observation suggests that the absolute value of the $\zeta$-potential does not correlate with contact activation. Other parameters, particularly a certain quantity of highly charged functional groups on the membrane surface, may be involved in this phenomenon.

Conclusions

The modification of PES with a polyelectrolyte additive results in an improved selective permeability for LMW proteins. PES achieves an efficiency of LMW protein removal in haemodialysis mode which matches results rather observed in more convective procedures, such as on-line post-dilution haemodiafiltration. Together with an optimum biocompatibility, which is most likely a result of enhanced mediator elimination, PES may contribute to adequate dialysis therapy preventing or at least retarding dialysis-related long-term complications in ESRD.

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