Effect of dialysis flux and membrane material on dyslipidaemia and inflammation in haemodialysis patients

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Abstract

Background. Dyslipidaemia, inflammation and oxidative stress are prominent risk factors that potentially cause vascular disease in haemodialysis patients. Dialysis modalities affect uraemic dyslipidaemia, possibly by modifying oxidative stress, but the effects of dialyser flux and membrane material on atherogenic remnant particles and oxidized low-density lipoproteins (LDL) are unknown.

Methods. We performed a randomized crossover study in 36 patients on haemodialysis to analyse the effect of dialyser flux and membrane material on plasma lipids, apolipoproteins and markers of inflammation and oxidative stress. Stable patients on low-flux dialysis with polysulphone for ≥6 weeks were assigned to high-flux polysulphone or high-flux modified cellulose with similar dialyser surface area and permeability characteristics and crossed over twice every 6 weeks.

Results. Thirty patients completed the study per protocol. Treatments with high-flux polysulphone and modified cellulose lowered serum triglyceride (by 20% and 10%, respectively; \( P < 0.05 \)) and remnant-like particle cholesterol by 32% (\( P < 0.001 \)) and 11% (NS) after the first 6 weeks of treatment. Oxidized LDL decreased significantly with high-flux polysulphone, but not with modified cellulose. Apolipoproteins CII and CIII were reduced, whereas the ratio CII/CIII was increased (all \( P < 0.05 \)). Acute-phase proteins and LDL and high-density lipoprotein cholesterol remained unaffected.

Conclusions. This randomized crossover study demonstrates a potent effect of high-flux haemodialysis on uraemic dyslipidaemia. Polysulphone membrane material showed superiority on oxidatively modified LDL, an indicator of oxidative stress in haemodialysis patients.

Keywords: acute-phase proteins; apolipoproteins; high-flux haemodialysis; lipids; oxidative stress; polysulphone

Introduction

Atherosclerotic cardiovascular disease is the leading cause of death in haemodialysis patients [1]. Among cardiovascular risk factors, uraemic dyslipidaemia, characterized by hypertriglyceridaemia, elevated remnant lipoprotein particles and low serum high-density lipoprotein cholesterol (HDL-C) may play a role. Profound alterations in the apolipoprotein profile are frequently encountered, including elevated levels of apolipoprotein CII (apo-CII) and CIII (apo-CIII) [2]. In addition, inflammation and oxidative stress potentially contribute to the accelerated atherosclerosis seen in these patients.

Recent data from clinical studies imply that the quality of haemodialysis membranes may have an impact on the extent of dyslipidaemia and that dialysis with high-flux synthetic membranes could attenuate hyperlipidaemia [3–8]. Such possible beneficial effects could be related either to dialysis membrane properties per se or to increased convective transport of large solutes with high-flux dialysis. Not all studies reported beneficial effects of high-flux haemodialysis on lipid abnormalities, but revealed at least a reduction in triglyceride levels [9]. Another study showed no differences of serum lipid or apolipoprotein levels when high-flux was compared with low-flux dialysis [10]. However, the membrane material differed between the dialysers, suggesting a potential influence of the membrane properties.

Here we addressed the question whether a new high-flux polysulphone membrane [11], allowing high middle molecular weight solute clearance, has an impact on the uraemic dyslipidaemia and acute-phase proteins in haemodialysis patients in comparison...
to a modified cellulose dialysis membrane with similar characteristics.

Subjects and methods

Patients

Thirty-six stable haemodialysis patients (22 men, 14 women) from one dialysis centre, who were eligible and gave consent, were randomized during March 2001 in this study. Six patients dropped out during the course of the study due to hospital admission \( (n=2) \), infection \( (n=1) \) or personal decision \( (n=3) \). Per-protocol analysis was carried out in 30 patients. The characteristics of the patients are given in Table 1. Causes of renal insufficiency were chronic glomerulonephritis \( (n=11) \), renal insufficiency of unknown origin \( (n=6) \), small kidneys of unknown origin \( (n=5) \), small kidneys of unknown origin \( (n=6) \), hereditary renal disease \( (n=3) \) and others \( (n=5) \). Eight patients were on lipid-lowering treatment, which was kept constant during the entire study period. Sixty-seven per cent of the patients received erythropoietin at a mean dose of 67 U/kg body weight/week. Iron was administered in 77% of the patients, mostly by intravenous administration. The study was conducted according to the principles of the declaration of Helsinki of 1964, as last revised in Edinburgh in October 2000, and was approved by the local ethics committee. All participating patients gave written informed consent.

Procedures

Upon entry into the study, a 6 week baseline period was recorded in all patients using a low-flux polysulphone dialyser (F7 HPS; Fresenius Medical Care, Bad Homburg, Germany) with a surface area of 1.6 m² (Figure 1). Thereafter, patients were randomized to group A or B. Group A continued haemodialysis treatment with the modified cellulose high-flux membrane CT 210G (Baxter Healthcare, Deerfield, IL, USA), whereas patients of group B continued treatment with polysulphone-based high-flux dialysis (FX100 Helixon®; Fresenius Medical Care, Bad Homburg, Germany). According to the protocol, patients of groups A or B were crossed over twice after 6 week treatment periods (Figure 1). A crossover design has been chosen to evaluate intraindividual effects of dialysers in the same patient. The double crossover, each high-flux dialyser type followed the other one, thereby assessing not only the effect of high-flux vs low-flux, but also to what extent the order of dialysers has an impact on the level of the lipid-lowering effect.

Blood was drawn after a 12 h overnight fast at the mid-week dialysis session during the last week of each treatment period before dialysis and heparinization was installed. Post-dialytic blood sampling was carried out for determination of dialysis dose [12]. Samples were centrifuged immediately \( (2,000 \text{ g}, 4 \degree \text{C}) \) and the supernatant was stored at \(-80 \degree \text{C}\) until analysis. Mean dialysis treatment time was 263 ± 21 min and blood flow ranged between 240 and 300 ml/min. Ultrafiltration rates and dialysate flow \( (500 \text{ ml/min}) \) were set according to the individual needs of the patients. It should be noted that all patients were routinely dialysed with machines equipped with the Diasafe® system, to effectively remove bacteria and endotoxins from the dialysis fluid [13]. Thereby, the influence of endotoxins on parameters of interest was minimized. All dialysis treatment parameters were strictly kept constant throughout the study. Heparinization \( (26.5 \text{ IU/kg/h}) \) and ultrafiltration volume did not differ between the study periods of the individual patient. Bicarbonate dialysate was used throughout.

Sample analysis

All samples were assayed together to minimize interassay biases. Urea, haemoglobin (Hb), glycosylated haemoglobin (HbA1c), albumin, total protein, fibrinogen and high-sensitive C-reactive protein (CRP) were measured with standard methods. Kt/V was calculated with the urea values determined before and after haemodialysis [12]. Serum total cholesterol and triglyceride concentration were measured using enzymatic methods (CHOD-PAP and GPO-PAP, respectively; Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula and HDL-C was determined after precipitation with phosphotungstic acid/magnesium chloride. Apolipoprotein B was measured by nephelometry. Apolipoproteins CII and CIII were determined by radial immunodiffusion technique (Daiichi Pure Chemicals, Tokyo, Japan). Measurement of remnant-like particle cholesterol (RLP-C) was done by a commercially available immunoseparation assay (Otsuka America Pharmaceuticals, Rockville, MD, USA). Oxidized LDL was determined by enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden).

Statistical analysis

Data of lipid parameters for each membrane type were analysed separately with respect to the treatment order. Data in tables are expressed as means ± SD. In figures data are given as means ± SEM. According to normal or non-normal distribution of data either the parametric t-test or the non-parametric Wilcoxon’s signed rank test for paired data were used. If applicable, an analysis of variance or the Kruskal–Wallis H-test was used. Differences were considered to be statistically significant if the \( p \)-value was < 0.05. All analyses were performed using the SPSS program package (SPSS Inc., Chicago, IL, USA).

**Fig. 1.** Study design and schedule of assessments. Patients treated for 6 weeks with a low-flux dialyser (F7 HPS) were randomized to group A (CT 210G dialyser) and group B (FX100 dialyser) and were crossed over twice after 6 week treatment periods.

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<table>
<thead>
<tr>
<th>Group A</th>
<th>F7 HPS</th>
<th>CT210G</th>
<th>CT210G</th>
<th>CT210G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>FX100</td>
<td>FX100</td>
<td>FX100</td>
<td></td>
</tr>
</tbody>
</table>

Week | -6 | 0 | 6 | 12 | 18 |
**Results**

Patients (12 female, 18 male; mean age: 61±12 years) were on maintenance haemodialysis treatment for ≥6 months (mean: 72±53 months). Baseline characteristics of patients randomized into groups A or B are depicted in Table 1. Patients of the two groups did not differ significantly from each other in regard to age, body mass index (BMI) and months on haemodialysis. BMI did not change during the study. Four patients of group A and three patients of group B had diabetes mellitus. Eight out of 30 received a constant dose (10 mg/day) of simvastatin or atorvastatin (five patients in group A and three in group B). Heparin administration was adjusted individually and kept constant throughout the study. The crossover design allowed intra-individual comparison of parameters. Biochemical parameters, such as HbA1c, serum total protein, albumin, fibrinogen and CRP, were not different between the two groups of patients at baseline (Table 1) and did not change significantly throughout the study. Haemoglobin (groups A and B: 11.9±1.1 g/dl and Kt/V (group A: 1.38±0.16; group B: 1.43±0.23) were also not different at baseline but increased significantly (group A: 12.4±1.1 g/dl, 1.58±0.16; group B: 12.4±1.3 g/dl, 1.52±0.26; P<0.05 for Hb, P<0.01 for Kt/V) when dialysis modality was changed from low-flux to high-flux treatment.

**Serum lipids and remnant cholesterol**

Six weeks of high-flux haemodialysis decreased serum triglyceride levels significantly (Table 2) as compared with baseline low-flux treatment. The effect was slightly more pronounced during treatment with polysulphone than with modified cellulose during the first 6 weeks as well as throughout the study (Figure 2). Serum total-, LDL- and HDL-cholesterol remained unchanged (Table 2).

Table 1. Patient characteristics and biochemical parameters at baseline

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=16)</th>
<th>Group B (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64±10</td>
<td>60±12.4</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>10/6</td>
<td>8/6</td>
</tr>
<tr>
<td>Diabetes mellitus (yes/no)</td>
<td>4/12</td>
<td>3/11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7±5.4</td>
<td>28.0±4.8</td>
</tr>
<tr>
<td>Time on haemodialysis (months)</td>
<td>70±58</td>
<td>75±58</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.9±1.1</td>
<td>11.9±1.1</td>
</tr>
<tr>
<td>Haemoglobin A1C (%)</td>
<td>6.0±1.2</td>
<td>6.3±1.4</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.1±0.3</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.1±0.4</td>
<td>7.2±0.3</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>4.9±0.9</td>
<td>4.6±1.2</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>1.8±1.9</td>
<td>1.4±2.0</td>
</tr>
<tr>
<td>*cKt/V</td>
<td>1.22±0.12</td>
<td>1.26±0.19</td>
</tr>
<tr>
<td>Use of statins (yes/no)</td>
<td>5/11</td>
<td>3/11</td>
</tr>
</tbody>
</table>

Treatment sequence group A: CT 210G – FX100 – CT 210G; treatment sequence group B: FX100 – CT 210G – FX100.

Table 2. Plasma concentration of lipid and apolipoprotein parameters after 6 weeks’ treatment with the respective dialyser

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>214±141</td>
<td>256±152</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>219±32</td>
<td>210±33</td>
</tr>
<tr>
<td>HDL-C</td>
<td>39.2±9.3</td>
<td>37.9±10</td>
</tr>
<tr>
<td>LDL-C</td>
<td>133±39</td>
<td>121±41</td>
</tr>
<tr>
<td>Ox-LDL</td>
<td>46.5±9.9</td>
<td>41.4±14.1</td>
</tr>
<tr>
<td>RLP-C</td>
<td>16.9±15.9</td>
<td>21.0±18.6</td>
</tr>
<tr>
<td>Apo-CII</td>
<td>6.6±2.4</td>
<td>6.6±2.7</td>
</tr>
<tr>
<td>Apo-CIII</td>
<td>17.2±6.3</td>
<td>18.0±5.4</td>
</tr>
</tbody>
</table>

Data are given as means±SD in mg/dl and ox-LDL in U/l. 
*P<0.05, **P<0.01 vs baseline.
the first 6 week period, was more pronounced with polysulphone (−32%; \( P < 0.01 \)) than with modified cellulose (−11%; not significant (NS)).

Oxidized LDL

Serum levels of oxidized LDL are given in Table 2. In general, oxidized LDL was lower during all three high-flux periods compared with low-flux haemodialysis. However, the reduction of oxidized LDL was statistically significant only with high-flux polysulphone haemodialysis (weeks 12 and 6, groups A and B, respectively) and not with high-flux modified cellulose (Figure 4). The reduction of oxidized LDL by high-flux dialysis appears to be a membrane permeability effect, whereas the difference between cellulose triacetate (CTA) and polysulphone is likely to result from biocompatibility of the membrane material.

Apolipoproteins

The concentrations of apo-CII and apo-CIII decreased during the study period with high-flux dialysis (Table 2). The decrease was in the order of −3% to −22%, attaining significance in the case of apo-CIII and apo-CII. The slope of the changes in concentration of the different apolipoproteins was similar with the two dialysers. Subsequently, the ratio apo-CII : apo-CIII increased in group A from 0.38 ± 0.07 to 0.42 ± 0.10, switching from low-flux to high-flux modified cellulose (\( P = \text{NS} \)), and in group B from 0.36 ± 0.08 to 0.44 ± 0.14, switching from low-flux to high-flux polysulphone (\( P = 0.001 \)). The concentration of apolipoprotein B did not change significantly throughout the study.

Discussion

Dyslipidaemia, inflammation and oxidant stress are prominent phenomena in end-stage renal disease (ESRD) which promote atherosclerosis. Several treatment approaches are currently proposed or are under investigation in order to decrease the burden of cardiovascular disease in the ESRD population [1]. This study approaches the issue by investigating recent developments in dialyzer biocompatibility on selected parameters of lipid metabolism, acute-phase proteins and oxidized LDL by using a detailed crossover protocol allowing control for dialyzer flux and surface area.

The results of the present study demonstrate that high-flux dialysis, independent of membrane material, strongly improved the concentration of serum triglycerides, the ratio of CII and CIII apolipoproteins and oxidized LDL. The main findings of the present study were that lowering of RLP-C by high-flux dialysis treatment was well beyond the effects achieved by treatment with statins [14]. In addition, superiority of the high-flux polysulphone membrane in decreasing the concentrations of oxidized LDL was demonstrated.

Chronic renal failure is characterized by disturbances in lipid and apolipoprotein metabolism causing increased levels of triglycerides, apo-CIII and RLP-C [2]. The decrease of triglyceride levels and the improvement of the apo-CII : apo-CIII ratio during 6 weeks of treatment is most likely the effect on the recovery of lipoprotein lipase activity. Lipoprotein lipase requires apo-CII to be active, but being also under control through its inhibitor apo-CIII [15]. Apparently, cholesterol-rich lipoprotein particles were not affected and no significant effects on serum total and LDL cholesterol was observed.

The beneficial effect of high-flux membranes, such as polysulphone or CTA, upon triglyceride levels has been shown in several studies during recent years [3–9]. Dumler et al. [16] found a significant decrease of triglycerides and total cholesterol during 6 months of high-flux polysulphone haemodialysis. Josephson et al. [3] reported improved hypertriglyceridaemia in patients dialysed with high-flux (polysulphone and modified cellulose) vs low-flux cellulose during 8 months of treatment. A parallel group comparison between low-flux cellulose and high-flux polysulphone demonstrated a significant improvement of triglyceride and cholesterol concentrations after a single treatment [4] and during long-term use [5]. To separate between the effect of membrane permeability and biocompatibility, Goldberg et al. [6], Merello Godino et al. [8] and House et al. [9] focused on the flux difference of polysulphone dialysers. At first the results appeared conflicting, because Goldberg et al. showed that high-flux polysulphone dialysis led to a significant improvement of triglyceride and total cholesterol concentrations after 1 month of treatment, as also confirmed by Merello Godino et al. after 6 months of high-flux treatment. House et al. could not confirm these results over a treatment period of 3 months and found non-significant decreases of triglyceride in the high-flux as compared with the low-flux group. The present randomized, crossover study is addressing the influence of membrane permeability (low-flux polysulphone vs high-flux polysulphone/modified cellulose) and membrane biocompatibility without flux influence (polysulphone vs modified cellulose), separately. As the capacity of a membrane to pass molecules of a certain size is independent from the

Fig. 4. Percentage difference of oxidized LDL levels from baseline in two groups of patients using a crossover design. Treatment in group A was started with CT210G (hashed bars), whereas in group B FX100 (filled bars) was used initially. *\( P < 0.05 \) vs baseline. Data are given as means ± SEM.

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Week 6</th>
<th>Week 12</th>
<th>Week 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized LDL</td>
<td>0.38 ± 0.07</td>
<td>0.36 ± 0.08</td>
<td>0.36 ± 0.08</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>Apo-CII</td>
<td>0.42 ± 0.10</td>
<td>0.44 ± 0.14</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.10</td>
</tr>
<tr>
<td>Apo-CIII</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.10</td>
</tr>
</tbody>
</table>
actual membrane surface area, the difference of surface between low-flux and high-flux dialysers used in this study is not considered to influence to a relevant extent—the effects attributed to membrane permeability. Clearly, patients treated with high-flux membranes showed a significantly improved lipid and apolipoprotein profile after 6 weeks of treatment.

Another major difference between permeability categories was the significant reduction of RLP-C. The remnant particles are products of partially catabolized chylomicrons and very low-density lipoproteins from which triglycerides have been hydrolysed by the action of lipoprotein lipase and hepatic lipase. Indeed, triglyceride significantly correlated with RLP-C (r = 0.89), as also demonstrated previously by Hirany et al. [17]. The levels of RLP-C could be reduced through switching from low-flux to high-flux haemodialysis. However, RLP-C levels were still 3-fold higher than levels in healthy controls [18]. Looking at the sequence of high-flux dialysers administered to the groups of patients, it appears that not only the permeability of a membrane but also the membrane biocompatibility has an impact on RLP-C levels. In group A, the RLP-C concentration decreased constantly over treatment periods, independent of the high-flux dialyser used. In group B, the RLP-C level rose again during high-flux haemodialysis with modified cellulose, after achieving statistical significance with high-flux polysulphone treatment. Assuming that a high-flux compared with a low-flux membrane may have more adequately removed circulating inhibitors of lipoprotein lipase, then the RLP-C difference between the two high-flux dialysers, modified cellulose and polysulphone, still points to a possible differential release of cytokines and other inflammatory mediators that may have had an influence on the lipid profile independent of membrane flux. The influence of other, external inflammatory stimuli has been minimized, as all patients have been treated with double-filtered dialysis fluid.

Two investigations showed that lipids and apolipoproteins are subject to oxidation in uraemic patients, due to prolonged circulation or intradialytic modifications [19]. Subsequently, LDLs are exposed to oxidation by free radicals, enhancing their atherogenicity [20]. In the present study, levels of oxidized LDL were significantly reduced after 6 weeks of treatment with high-flux polysulphone haemodialysis, an effect that could not be achieved with high-flux modified cellulose dialysis.

The effects achieved with high-flux dialysis on RLP-C lowering were in the range of 27–41%. This is an at least comparable order of magnitude as achieved with pharmacological lipid-lowering therapy [14]. A logical conclusion from this data would be the recommendation of high-flux dialysis for all patients in order to achieve outcome benefits mediated through reduction of the atherogenic burden. A reduction of atherogenic triglyceride-rich remnant particles and oxidized LDL of such magnitude should translate into improved cardiovascular outcomes, at least as demonstrated in the general population. On the other hand, it should be noted that the HEMO Study, comparing high-dose high-flux vs low-flux dialysis, provided negative secondary outcome data of first hospitalization for cardiac causes [21]. In respect to these findings, one may ask about the potential benefits following improvement of the risk factors studied here. Similar conditions in respect to flux and dialysis dose occurred in the HEMO Study and with this study. It remains to be studied whether risk factors different from dyslipidaemia are more important in the development of vascular disease in populations characterized by pronounced inflammation. This condition typically reverses established causalities of known cardiovascular risk factors, such as cholesterol or obesity, in their prediction of outcome [22]. It is also of importance to note that high-flux dialysis, although in our study posed on top of already high-quality dialysis, had no influence on concentrations of acute-phase proteins in plasma. Levels of two positive acute-phase proteins, CRP and fibrinogen, and one negative acute-phase protein, albumin, did not change throughout the 18 weeks of controlled treatment. Interestingly, statins that lower remnant lipoprotein concentrations and oxidized LDL also decrease CRP. However, statins also lower native LDL-C, whereas high-flux mainly impacts on triglyceride-rich lipoproteins. It remains to be studied whether the two strategies should be combined. The present data are of importance in respect to the interpretation of future data coming from large-scale lipid-lowering trials, once these trials are finalized.

In conclusion, this study demonstrates a significant effect of high-flux haemodialysis on uraemic dyslipidaemia. In addition, a new membrane dialyser made from modified polysulphone showed a major effect on oxidized LDL, an indicator of oxidative stress. These results suggest that high-flux polysulphone dialysis may be of benefit in reducing the atherogenic risk in haemodialysis patients.

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References

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