Dialysis filter type determines the acute effect of haemodialysis on endothelial function and oxidative stress

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Abstract

Background. Endothelial function of large arteries is impaired in chronic haemodialysis patients and oxidative stress due to the dialysis procedure has been suggested as a causal factor. However, it is not clear whether different types of dialysis membranes affect endothelial function differently. Therefore we determined endothelium-dependent, flow-mediated dilatation (FMD) of the brachial artery as well as markers of oxidative stress immediately before and after haemodialysis (HD) with either a cellulosic cuprophane or a synthetic polysulphone dialyser in a blinded, randomized, cross-over study.

Methods. Twelve haemodialysis patients (age 55 ± 3 years, time on dialysis 20 ± 2 months, mean fluid change/C0 1782 ± 21 ml, systolic blood pressure 139 ± 75 mmHg) were included. Using a multi-gate-pulsed Doppler system (echo-tracking device) brachial artery FMD and nitroglycerine-induced, endothelium-independent vasodilatation (NMD) were measured. Patients were randomized to HD with either a polysulphone or a cuprophane membrane and were crossed over to the other filter. Investigators were blinded to the type of membrane used. Serum concentrations of oxidized LDL (oxLDL) and α-tocopherol as markers of oxidative stress were measured before and after each dialysis session.

Results. Data are given as mean ± SEM. Treatment with polysulphone filter HD did not significantly affect FMD (baseline 9.3 ± 2.0% vs after HD 9.6 ± 1.8%). After dialysis with a cuprophane membrane FMD decreased from 9.4 ± 2.1 to 7.4 ± 1.8% (P < 0.05). NMD was not significantly affected by HD irrespective of the membrane material used. Serum levels of oxLDL were not changed by either treatment; however, α-tocopherol concentrations fell significantly after dialysis with the cuprophane filter (baseline 18.0 ± 2.3 after HD 16.6 ± 1.3 μg/ml, P < 0.05), while α-tocopherol levels remained unchanged when the polysulphone membrane was used.

Conclusions. The type of dialysis filter membrane determines the acute effect of haemodialysis on arterial endothelial function. Differences in biocompatibility and oxidative stress may account for the observed differential effects, since the decrease of FMD after dialysis with a cellulosic cuprophane membrane—but not with a synthetic polysulphone membrane—was associated with a reduction in serum vitamin E.

Keywords: cuprophane; endothelial function; flow-mediated vasodilatation; haemodialysis; oxidative stress; polysulphone

Introduction

Endothelial dysfunction has consistently been observed in patients with end-stage renal disease and may contribute to the high cardiovascular morbidity and mortality in these patients [1,2]. A number of factors such as arterial hypertension, chronic fluid overload, uraemia, sympathetic activation and hyperparathyroidism have been implicated in impairment of the functional arterial vessel properties in patients with renal insufficiency [3–5]. Whether the dialysis procedure per se causes significant impairment of endothelial function is still a matter of debate [6–8]. While Miyazaki and co-workers [8] have reported an acute decrease in brachial artery endothelial function by dialysis with a cellulosic membrane, Hand et al. [7] observed an improvement of venous endothelial dysfunction by haemodialysis and suggest that endogenous inhibitors of nitric oxide synthase—that accumulate in end-stage renal disease—may be cleared by the dialysis procedure. We recently reported that haemodialysis with a biocompatible, synthetic polysulphone...
membrane did not cause significant acute effects on the endothelial function of the brachial artery [6]. It is conceivable that differences in biocompatibility and in the degree of oxidative stress associated with the dialysis procedure account for the discrepancies.

Activation of leukocytes and platelets as well as oxidation of lipids during haemodialysis have been suggested to play a role in the impairment of functional vessel-wall properties [9,10]. It has been shown that cellulosic membranes cause a higher degree of oxidative stress and are associated with untoward effects on granulocyte functions when compared to modern synthetic membranes like those used in polysulphone dialysers [10,11]. Whether these differences in biocompatibility of dialysers membranes are associated with significant differences in the effect of haemodialysis on arterial endothelial function is not clear.

The objective of the present study was therefore to compare acute effects of haemodialysis with cellulosic membrane (cuprophane) and synthetic polysulphone membrane on endothelial function as measured by brachial-artery flow-mediated dilatation in chronic dialysis patients. Moreover, the degree of oxidative stress associated with the dialysis procedure was studied by measuring oxidized low-density lipoproteins (LDL) and \( \alpha \)-tocopherol (vitamin E) serum levels as parameters of the oxidative balance.

**Subjects and methods**

**Patients**

We performed a blinded cross-over study which was approved by the ethical committee of the University of Münster School of Medicine. All patients gave their written informed consent. Eligible patients were between 18 and 65 years of age and on maintenance haemodialysis. Patients were included if coronary artery disease, heart failure, diabetes mellitus and visible plaques on high-resolution ultrasound of the carotid arteries could be excluded.

A total of 12 patients presenting at the dialysis centre of the University of Münster were recruited. Patients were randomly assigned to haemodialysis with either a synthetic low-flux polysulphone dialyser (F6 HPS, surface area 1.3 m\(^2\), steam-sterilized, Fresenius, Bad Homburg, Germany) or a cellulosic, low-flux cuprophane dialysis filter (Alwall GFS 12, surface area 1.3 m\(^2\), steam-sterilized, Gambro, Hechingen, Germany) and were crossed over to the other membrane the next dialysis session 2 days later. The dialysis duration was standardized with 4 h, the interval between randomization and the first dialysis session was the same in all patients. Vessel-wall properties and parameters of oxidative stress were studied before and after the haemodialysis session. Vessel-wall measurements were taken within 20 min after the end of the haemodialysis session. No i.v. medication apart from heparin was given during haemodialysis. Heparin doses were standardized and identical for both sessions with 2,000 U bolus at the beginning and 1,000 U during each hour of the following 4 h.

Table 1 shows clinical data of the patients. All patients were receiving regular haemodialysis three times weekly for 4 h. Four patients were hypertensive and were treated with ACE inhibitors (n = 2) or calcium antagonists in combination with an ACE inhibitor (n = 2). Antihypertensive medication was not changed during the study. All antihypertensive or vasoactive drugs were paused for a minimum of 24 h before the study of vessel-wall properties.

**Measurement of flow-mediated and nitroglycerine-induced vasodilatation**

All patients were studied by the same investigator who was blinded to the type of filter membrane used. All investigations were performed between 8 a.m. and 1 p.m. Only patients without an arteriovenous fistula or previous vascular surgery on the right arm were included in the study. Following blood sampling, blood pressure, flow-mediated and nitroglycerine-mediated vasodilatation of the brachial artery were measured as described earlier [1,6]. Briefly, the end-diastolic diameter of the right brachial artery 5 cm proximal of the elbow was studied in a longitudinal projection using a 7.5 MHz linear array transducer and a multigate-pulsed Doppler system with an ECG trigger (Vessel Wall Track System 2, Pie Medical Equipment BV, Maastricht, The Netherlands). Vessel-wall movements were monitored based on low-frequency Doppler signals originating from the sample volumes coinciding with the anterior and posterior wall. The Doppler signals in M-mode were temporarily stored and analysed by a personal computer system. The system allows the assessment of the relative change of major peripheral artery diameter as a continuous function of time with an accuracy of ~0.5%. Using this system, the end-diastolic diameter of the brachial artery was determined at five consecutive cardiac cycles and the results were averaged. The coefficient of variation for end-diastolic diameter at our centre is below 5% [1,6].

After three measurements at baseline had been taken, a forearm cuff was inflated at 300 mmHg for 4 min at least 10 cm distal the site of ultrasound measurement. During the last minute of cuff inflation and 1, 2, 3, 5, 7 and 10 min after cuff release further measurements were taken. Additionally, brachial-artery blood flow at baseline and during the initial 15 s of reactive hyperaemia was estimated using pulsed Doppler, and the degree of reactive hyperaemia as a percentage of the basal blood flow was calculated.

Fifteen minutes after cuff release, when vessel diameter had returned to baseline values, 400 \( \mu \)g of glycerol trinitrate

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**Table 1. Clinical and biochemical characteristics of 12 patients on maintenance haemodialysis using cuprophane and polysulphone filter membranes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.9 ± 3.0</td>
<td>41–64</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/5</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>22.8 ± 1.1</td>
<td>19.5–25.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>219 ± 9</td>
<td>178–264</td>
</tr>
<tr>
<td>Total triglycerides (mg/dl)</td>
<td>201 ± 8</td>
<td>132–248</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.6 ± 1.2</td>
<td>9.6–12.5</td>
</tr>
<tr>
<td>Parathyroid hormone (pg/ml)</td>
<td>112 ± 9</td>
<td>48–153</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.85 ± 0.2</td>
<td>0.5–7.2</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>20 ± 2</td>
<td>15–28</td>
</tr>
<tr>
<td>Ultrafiltration (ml) cuprophane</td>
<td>1702 ± 21</td>
<td>1640–1950</td>
</tr>
<tr>
<td>Ultrafiltration (ml) polysulphone</td>
<td>1781 ± 23</td>
<td>1600–2040</td>
</tr>
</tbody>
</table>
were administered sublingually and further scans of the brachial artery were taken after 1, 2, 3 and 5 min. Flow-mediated vasodilatation was calculated as the maximum absolute and relative increase in brachial-artery end-diastolic diameter during reactive hyperaemia. Nitroglycerine-induced vasodilatation was calculated accordingly as the maximum increase in artery diameter after sublingual application of glycerol trinitrate.

**Laboratory analysis**

Blood samples were drawn immediately before the start of dialysis and 5 min before the end of dialysis. Samples were centrifuged immediately and frozen at −20°C. Vitamin E (α-tocopherol) was measured using high performance liquid chromatography (HPLC). Oxidized LDL levels in EDTA-plasma samples were measured with the oxidized LDL capture ELISA kit (Mercodia, Uppsala, Sweden) utilizing the specific murine monoclonal antibody 4E6 described by Holvoet and co-workers [12]. Briefly, the ELISA assay is a solid two-phase two-site enzyme immunoassay which is based on a direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. The colorimetric end-point is read spectrometrically at 450 nm.

**Statistics**

Data are expressed as mean ± SEM. The effects of haemodialysis treatment on the measured variables were tested by repeated measures ANOVA and post-hoc comparisons (planned contrasts). Statistical significance was assumed at $P < 0.05$.

**Results**

Table 2 shows blood pressure, heart rate and arterial vessel-wall parameters before and after haemodialysis with a cuprohane and a polysulphone dialyser. There were no significant changes in blood pressure, heart rate or brachial-artery diameter with dialysis using either membrane.

Flow-mediated vasodilatation was significantly reduced by dialysis with a cuprohane membrane from $9.4 ± 1.8\%$ before the dialysis session to $7.4 ± 1.7\%$ after dialysis (Figure 1, Table 2). When the polysulphone dialyser was used, no significant change in flow-mediated vasodilatation was observed ($9.3 ± 1.9\%$ before and $9.6 ± 1.8\%$ after dialysis). Nitroglycerine-induced, endothelium-independent vasodilatation remained unchanged by dialysis with either membrane.

Increase in blood flow after release of the occlusion was estimated by pulsed Doppler and was not significantly different between measurements before and after dialysis with either membrane.

Concentrations of oxidized LDL were not influenced by dialysis with cuprohane or polysulphone membranes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before POL</th>
<th>After POL</th>
<th>Before CUP</th>
<th>After CUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>139 ± 7</td>
<td>136 ± 7</td>
<td>136 ± 8</td>
<td>134 ± 8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75 ± 4</td>
<td>76 ± 5</td>
<td>76 ± 4</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>Heart rate (l/min)</td>
<td>77 ± 5</td>
<td>77 ± 8</td>
<td>78 ± 8</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Brachial diameter (mm)</td>
<td>45.5 ± 0.21</td>
<td>4.53 ± 0.82</td>
<td>4.51 ± 0.72</td>
<td>4.49 ± 0.87</td>
</tr>
<tr>
<td>Flow-mediated vasodilatation (mm)</td>
<td>0.42 ± 0.06</td>
<td>0.41 ± 0.04</td>
<td>0.42 ± 0.05</td>
<td>0.31 ± 0.08$^{a,b}$</td>
</tr>
<tr>
<td>Nitroglycerine-induced dilatation (mm)</td>
<td>0.67 ± 0.12</td>
<td>0.59 ± 0.13</td>
<td>0.62 ± 0.21</td>
<td>0.66 ± 0.16</td>
</tr>
<tr>
<td>Nitroglycerine-induced dilatation (%)</td>
<td>14.6 ± 0.6</td>
<td>14.4 ± 0.8</td>
<td>14.4 ± 1.0</td>
<td>14.8 ± 0.9</td>
</tr>
<tr>
<td>Oxidized LDL concentrations (mg/l)</td>
<td>11.1 ± 0.8</td>
<td>11.5 ± 0.9</td>
<td>11.2 ± 0.7</td>
<td>11.3 ± 0.8</td>
</tr>
</tbody>
</table>

$^aP < 0.05$ before vs after dialysis; $^bP < 0.01$ for session × time interaction.

**Fig. 1.** Relative flow-mediated vasodilatation (FMD) of the brachial artery in 12 patients before and after haemodialysis with a polysulphone (POL) and a cuprohane (CUP) membrane dialyser. Bars indicate the mean values. $P < 0.01$ for session × time interaction.
However, plasma concentrations of α-tocopherol were significantly decreased by dialysis with cuprophane dialysers, while dialysis with polysulphone membranes had no effect on α-tocopherol levels (Table 2, Figure 2). These data suggest that dialysis with the biocompatible low-flux polysulphone membrane cause less vitamin E-consuming oxidative stress and less impairment of endothelial function than dialysis with the cellulosic cuprophane membrane dialyser.

Discussion

The major finding of this study was that haemodialysis with a cellulosic cuprophane membranes resulted in a significant blunted flow-mediated dilatation of the brachial artery, whereas dialysis with a synthetic polysulphone dialyser had no such effect on endothelial function. The decrease in endothelial function observed with cuprophane dialyser was associated with a moderate fall in serum α-tocopherol concentrations as a marker of an increase in oxidative stress which was not seen with a polysulphone membrane.

To the best of our knowledge, this is the first study comparing the effects of cellulosic and synthetic membranes on endothelial function in haemodialysis patients. We did not find any changes in nitroglycerine-induced vasodilatation, a parameter of endothelium-independent arterial function, after dialysis by either membrane, suggesting that the observed changes in flow-mediated vasodilatation of the brachial artery were indeed due to alteration in endothelial function. Moreover, the end-diastolic diameter of the brachial artery and the increase in blood flow during reactive hyperaemia were not significantly altered during the dialysis with either membrane, indicating that the degree of shear stress in the brachial artery was comparable. Our data suggest that endothelial dysfunction may be improved by the use of biocompatible, synthetic membranes in chronic dialysis patients.

Since endothelium-mediated vasodilatation of peripheral and coronary arteries is closely related [13] and haemodialysis treatment is applied several times per week, our finding of blunted endothelial function of the brachial artery after dialysis with the cuprophane membrane may be of prognostic relevance. Although the changes in flow-mediated vasodilatation observed after dialysis were relatively mild, it is conceivable that the type of dialyser membrane and the degree of biocompatibility may impact on cardiovascular morbidity and mortality in patients on chronic haemodialysis treatment.

Dialysis-induced oxidative stress is considered a major factor contributing to the high cardiovascular morbidity and mortality in patients on chronic haemodialysis treatment, and therapeutic strategies, including the use of synthetic, biocompatible membranes, have been suggested to minimize the harmful consequences of a pro-oxidative state during dialysis [11]. However, the measurement and quantification of oxidative stress remains a difficult issue in this setting.

It has been suggested that the oxidation of low-density lipoproteins plays a key role in the biological process that results in the development of atherosclerotic lesions [14]. Moreover, oxidized LDL has been reckoned to contribute directly to endothelial dysfunction by impairing the signal transduction between the endothelial cell surface receptors and nitric oxide (NO) production and inhibition of the NO synthase [15]. However, ‘reference ranges’ or kinetics of oxidized LDL during acute oxidative stress, such as during a haemodialysis session, have not yet been established. Recently, Miyazaki and co-workers [8] reported a 35% increase of plasma oxLDL concentrations after a single session of haemodialysis with a cellulosic membrane. In our study, plasma levels of oxLDL did not show any significant changes during dialysis therapy with either membrane. There are two possible explanations for the stable oxLDL levels found in our study. First, no differences in oxidative stress exerted by the membranes were present. However, this would not agree with the significant
decrease in vitamin E (z-tocopherol) that was observed after dialysis with cuprophane but not with the polysulphone membrane. Secondly, oxidized LDL might have been cleared from the plasma before samples were taken at the end of dialysis. There is some evidence from animal experiments that the latter hypothesis might hold true. Ling and co-workers showed that radioactive-labelled oxidized LDL is rapidly cleared from the bloodstream in mice [16]. Over 90% of the injected material was cleared within 5 min, mostly by the liver. Based on these data, the rapid clearance of newly formed oxLDL sheds doubt on whether this parameter is indeed a reliable marker of acute systemic oxidative stress in the haemodialysis setting.

On the other hand, levels of z-tocopherol decreased significantly after dialysis with the cellulosic cuprophane membrane in our study, whereas no such changes were observed after dialysis with polysulphone. Vitamin E is a reliable marker of systemic oxidation and an inverse correlation with the duration of dialysis treatment has been observed for plasma z-tocopherol levels but not for other antioxidants [17]. It is therefore conceivable, that the small but significant decrease in plasma vitamin E levels observed after dialysis with cuprophane argues for more oxidative stress as compared to dialysis with polysulphone membranes. However, given the relatively mild decrease in z-tocopherol it is questionable whether this is a clinically meaningful change; other direct and indirect markers of oxidative stress may clarify this issue. We did not study other indices of oxidative stress; this limitation has to be considered when interpreting our results. It remains a hypothesis whether relevant differences in the exerted oxidative stress were present in our study and also whether these changes are related to the deleterious effect of cuprophane dialysis on endothelial function that were observed. Other, oxidation-independent mechanisms may contribute to the different effects on arterial function of cuprophane and polysulphone membranes, for example differences in activation of the complement system by the dialyser membranes. It has been shown, that haemodialysis with cuprophane membranes is associated with greater activation of the alternative pathway of complement than dialysis with a more biocompatible membrane, and that after passage through a cuprophane dialyser more active complement fragments are present [18]. Moreover, haemodialysis with a cuprophane membrane induces neutropenia and expression of the granulocyte adhesion receptor Mac-1 (CD11b/CD18), while haemodialysis with non-complement-activating membranes does not [19]. Finally, Linnenweber and co-workers [20] demonstrated recently that impaired function of peripheral blood mononuclear cells in patients on cuprophane haemodialysis could be normalized when patients were switched to polysulphone. The influence of dialyser membranes on function of mononuclear cells and on stimulation of adhesion molecules may also alter leukocyte–endothelium interactions and endothelial function. It has been shown that cuprophane dialysis severely impairs rolling, adhesion and transendothelial migration of leukocytes in an experimental setting [21]. However, we did not study the cellular mechanisms of blood–membrane interactions.

Moreover, when interpreting the relatively moderate acute changes in endothelial function that we observed, it has to be considered that apart from haemodialysis per se a number of other factors such as chronic fluid overload, hyperparathyroidism, hypercholesterolaemia and sympathetic activation may contribute to endothelial dysfunction in patients with end-stage renal failure [3,4].

Our data are in accordance with data from Miyazaki and co-workers, who reported a significant reduction in flow-mediated dilatation after a single session of haemodialysis using a cellulosic membrane in patients on maintenance dialysis [8]. In this study, the acute effect on endothelial function was blunted by the use of a vitamin E-coated dialyser, which apparently caused less oxidative stress compared to a standard cellulosic membrane. Here we show that the detrimental effects on endothelial function seen with cellulosic membranes may be prevented by a polysulphone dialyser. This is in accordance with a previous study in which we found no deleterious effect of a haemodialysis session on endothelial function when a polysulphone filter was used [6].

Based on our findings we conclude that differences in membrane material and thus in the biocompatibility of the dialyser impact on endothelial function during haemodialysis. While dialysis per se with a synthetic polysulphone membrane does not have an acute effect on flow-mediated vasodilatation, dialysis with a cellulosic cuprophane membrane is associated with a small but significant decline in endothelial function. This effect is possibly due to increased oxidative stress, as shown by a moderate decrease in vitamin E levels, and might contribute to the cardiovascular morbidity in long-term haemodialysis patients. However, whether regular dialysis with a cellulosic membrane is associated with a higher cardiovascular event rate than dialysis with a synthetic membrane remains to be elucidated by further prospective long-term studies involving large numbers of patients.

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Conflict of interest statement. None declared.

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Dialysis membranes and endothelial function


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