Original Article

Effect of dialyser membrane pore size on plasma homocysteine levels in haemodialysis patients

An S. De Vriese1, Michel Langlois2, Dirk Bernard2, Inge Geerolf1, Lut Stevens1, Johan R. Boelaert1, Mario Schurgers1 and Erve Matthys1

Abstract

Background. Hyperhomocysteinaemia is a putative risk factor for atherothrombotic cardiovascular disease in the haemodialysis population. High-dose vitamin B therapy does not entirely normalize elevated plasma total homocysteine (tHcy) levels in haemodialysis patients. Alternative therapies to reduce tHcy further are therefore required. Modifications of the dialysis regimen may result in a better removal of Hcy. We examined the effect of dialyser membrane pore size on tHcy levels in vitamin-replete chronic haemodialysis patients.

Methods. Forty-five haemodialysis patients were dialysed during 4 weeks with a low-flux, a high-flux and a super-flux membrane, in random order. Pre-dialysis tHcy was determined at baseline and every 4 weeks. In 18 patients, plasma tHcy before and after dialysis and dialysate tHcy concentrations were measured.

Results. Pre-dialysis tHcy decreased significantly during 4 weeks super-flux dialysis (−14.6 ± 2.8%), whereas it remained stable during high-flux (+0.5 ± 2.4%) and low-flux dialysis (+1.7 ± 3.2%). The homocysteine reduction ratio was not different for the three membranes: 0.39 ± 0.03 for the super-flux, 0.47 ± 0.02 for the high-flux and 0.39 ± 0.02 for the low-flux dialyser. The amount of Hcy recovered in the dialysate during a single dialysis session was also similar: 117.5 ± 3.6 μmol during super-flux, 95.3 ± 11.5 μmol during high-flux and 116.5 ± 11.6 μmol during low-flux dialysis.

Conclusion. Super-flux dialysis significantly lowers tHcy in chronic haemodialysis patients. Improved removal of middle-molecule uraemic toxins with inhibitory effects on Hcy-metabolizing enzymes, rather than better dialytic clearance of Hcy itself, may explain the beneficial effect of the super-flux membrane.

Keywords: dialyser membrane; haemodialysis; high-flux; low-flux; super-flux; total homocysteine (tHcy)

Introduction

Mild to moderate hyperhomocysteinaemia is observed in the large majority of patients with end-stage renal disease (ESRD) treated with chronic haemodialysis. Several prospective studies have identified hyperhomocysteinaemia as an independent risk factor for atherothrombotic cardiovascular disease in the haemodialysis population [1–3]. More recently, however, an inverse association between total homocysteine (tHcy) levels and cardiovascular complications was reported [4,5], possibly owing to confounding by malnutrition, inflammation and diabetes, which are all associated with lower tHcy levels as well as with an increased incidence of cardiovascular disease. These diverging results dictate the need to design interventional studies, in order to clarify the contribution of hyperhomocysteinaemia to the excess cardiovascular morbidity and mortality in the haemodialysis population.

Unfortunately, so far, no treatment regimen has succeeded in normalizing tHcy concentrations in the haemodialysis population. Although high-dose vitamin B supplementation markedly reduces tHcy, most patients maintain residual hyperhomocysteinaemia [6]. Further, attempts to lower tHcy in haemodialysis patients with betaine or N-acetylcysteine have been unsuccessful. A decrease of tHcy levels by 30–40% has been observed during a single haemodialysis session [7–10], with a rebound after ~8 h [9,10]. The question has arisen of whether the dialysis procedure could be modified to optimize Hcy removal. While high-flux dialysis did not portend a better reduction in tHcy concentrations than low-flux dialysis [11–13], dialysis with a super-flux membrane significantly lowered tHcy compared with a high-flux membrane [14]. Several of these studies, however, compared haemodialysis
membranes with different membrane material, thickness or surface area or did not match for vitamin intake. In the present study, we compared the effect of dialysis with a low-flux, high-flux and super-flux membrane with pore size as the only distinctive characteristic on tHcy levels in vitamin-replete chronic haemodialysis patients. In addition, dialytic Hcy removal was measured, in order to clarify potential differences between membranes.

Subjects and methods

Patients

Forty-five haemodialysis patients (20 males, 25 females) with a mean age of 69.0 ± 1.8 years (range 36–89) were randomly recruited from the AZ Sint-Jan AV haemodialysis unit. They were dialysed with a low-flux triacetate dialyser (Sureflux-L, Nipro, Osaka, Japan) during 4–5 h thrice weekly for a period of at least 3 months. Exclusion criteria were: acute illness, life expectancy <3 months, problems with vascular access, excessive interdialytic weight gains requiring regular additional dialyses and inability to provide informed consent. All patients received folic acid 5 mg, pyridoxine 50 mg and vitamin B12 12 µg orally thrice weekly during dialysis in order to ascertain compliance. Written informed consent was obtained. The study protocol was approved by the Ethical Committee of AZ Sint-Jan AV.

Study protocol

Patients were randomly assigned to one of three treatment regimens (Figure 1). They were dialysed during 4 weeks with a low-flux triacetate dialyser (Sureflux-150L, Nipro), a high-flux triacetate dialyser (FB-150U, Nipro) and a super-flux triacetate dialyser (Sureflux-150FH, Nipro), in random order. All three dialyser types have the same surface area and consist of triacetate, but have different pore size (Table 1). The dialysers were single-use only. Treatment duration, blood flow, dialysate flow, dialysate temperature and ultrafiltration profiles were determined by the attending nephrologist, but no changes were made during the study period.

Pre-dialysis blood samples were obtained at baseline and every 4 weeks, transported on ice, immediately centrifuged and stored at −20°C until testing. Patients ate their usual meals before sampling, and subsequent samples were obtained at roughly the same time of the day in each patient. In six randomly selected patients from each group, blood samples were obtained before and directly after a mid-week dialysis. During this dialysis session, dialysate samples were collected 1, 2, 3 and 4 h after the start of dialysis.

Biochemical analyses and calculations

Plasma tHcy concentrations were measured using a fluorescence polarization immunoassay (AxSYM, Abbott Laboratories, IL). Plasma vitamin B12 and erythrocyte folate levels were measured with the SimulTRAC-SNB radioimmunoassay kit (ICN Pharmaceuticals, NY). Plasma creatinine, urea and albumin concentrations were measured using standard automated clinical chemistry techniques (Modular, Roche Diagnostics, Mannheim, Germany). Albumin concentration in the dialysis fluid was assessed by kinetic immunoturbidimetry (Dade Behring, Marburg, Germany).

The homocysteine reduction ratio was calculated as (homocysteine_{pre-dialysis} − homocysteine_{post-dialysis})/homocysteine_{pre-dialysis}. The homocysteine removal rate at 1, 2, 3

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**Table 1. Dialyser characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Sureflux-150L</th>
<th>FB-150U</th>
<th>Sureflux-150FH</th>
</tr>
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<tbody>
<tr>
<td>Effective surface area, m²</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Inner diameter, µm</td>
<td>200</td>
<td>200</td>
<td>185</td>
</tr>
<tr>
<td>Membrane thickness, µm</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Pore size, Å</td>
<td>50</td>
<td>70</td>
<td>78</td>
</tr>
<tr>
<td>Ratio of open pores, %</td>
<td>63</td>
<td>70</td>
<td>84</td>
</tr>
<tr>
<td>Sieving coefficient β₂-microglobulin</td>
<td>0.36</td>
<td>0.88</td>
<td>1</td>
</tr>
<tr>
<td>Ultrafiltration coefficient, ml/h/mmHg</td>
<td>12.8</td>
<td>29.8</td>
<td>66.9</td>
</tr>
</tbody>
</table>
and 4 h after the start of dialysis was calculated based on the homocysteine concentration in the dialysate at that time point and the dialysate flow. The total amount of homocysteine recovered in the dialysate was calculated as the area under the curve of the homocysteine removal rate and the treatment time.

**Statistical analysis**

Data are presented as mean ± SEM. Non-parametrical statistical analyses were used because of the non-Gaussian distribution of tHcy. The Mann-Whitney U-test and the Wilcoxon’s rank test were applied as appropriate. An a priori level of α = 0.05 was used to indicate statistical significance.

**Results**

There were no baseline differences between the three groups with respect to age, creatinine, albumin, Kt/V, nPCR, plasma vitamin B₁₂, erythrocyte folate or baseline plasma tHcy levels (Table 2). Forty-three patients completed the study. One patient was withdrawn during dialysis with the super-flux membrane for a presumed reaction to the membrane, and another patient received a kidney transplant during the study period.

During 4 weeks dialysis with the low-flux and high-flux membranes, no significant changes in tHcy levels were observed (Figures 2 and 3). In contrast, 4 weeks dialysis with the super-flux membrane resulted in a significant decrease of the pre-dialysis tHcy concentration (Figures 2 and 3). Pre-dialysis tHcy was higher at the start of a super-flux treatment period, owing to the absence of a wash-out period before crossing over to another dialyser (Figure 1). The tHcy concentration after a particular treatment period is thus identical to the tHcy before a subsequent treatment period. Since low-flux and high-flux dialysis did not affect tHcy values, while super-flux decreased tHcy, the tHcy concentration after a low-flux or high-flux period is higher than after a super-flux period. Thus, patients are more likely to start a super-flux period with higher tHcy values.

The homocysteine reduction ratio was not significantly different between the groups: 0.39 ± 0.03 for the super-flux, 0.47 ± 0.02 for the high-flux and 0.39 ± 0.02 for the low-flux dialysate (P = NS). The amount of Hcy recovered in the dialysate during a single dialysis session was 117.5 ± 3.6 μmol during super-flux, 95.3 ± 11.5 μmol during high-flux and 116.5 ± 11.6 μmol during low-flux dialysis (P = NS). Albumin concentration in the dialysate of the low-flux and high-flux membranes was below the detection limit. In the dialysate of the super-flux membrane, 5.24 ± 0.40 g of albumin was recovered. Serum albumin did not change significantly during dialysis with the super-flux (3.66 ± 0.06 g/dl before, 3.62 ± 0.06 g/dl after), the high-flux (3.66 ± 0.07 g/dl before, 3.52 ± 0.07 g/dl after) or the low-flux (3.67 ± 0.06 g/dl before, 3.74 ± 0.07 g/dl after) membrane.

**Table 2. Baseline patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age, years</td>
<td>65.3 ± 3.3 (36-82)</td>
<td>69.2 ± 3.2 (48-89)</td>
<td>72.3 ± 2.9 (39-87)</td>
</tr>
<tr>
<td>Male/female</td>
<td>9/6</td>
<td>4/11</td>
<td>7/8</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>7.78 ± 0.57</td>
<td>8.15 ± 0.60</td>
<td>8.02 ± 0.42</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>3.60 ± 0.08</td>
<td>3.53 ± 0.10</td>
<td>3.50 ± 0.09</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.24 ± 0.1</td>
<td>1.41 ± 0.06</td>
<td>1.41 ± 0.05</td>
</tr>
<tr>
<td>nPCR</td>
<td>0.83 ± 0.07</td>
<td>0.94 ± 0.07</td>
<td>0.88 ± 0.03</td>
</tr>
<tr>
<td>Plasma vitamin B₁₂, pg/ml</td>
<td>828 ± 105</td>
<td>892 ± 103</td>
<td>901 ± 102</td>
</tr>
<tr>
<td>Erythrocyte folate, ng/ml</td>
<td>3232 ± 220</td>
<td>3435 ± 214</td>
<td>3293 ± 269</td>
</tr>
<tr>
<td>Plasma tHcy, μmol/l</td>
<td>22.06 ± 1.32</td>
<td>20.74 ± 1.36</td>
<td>20.05 ± 1.11</td>
</tr>
</tbody>
</table>
Discussion

In accordance with literature data [6], our haemodialysis population receiving a pharmacological dose vitamin B supplementation exhibited mild to moderate hyperhomocysteinaemia, with mean pre-dialysis levels of ~20 μmol/l. The salient observation of the present study is that dialysis with a super-flux membrane significantly lowered pre-dialysis tHcy levels, whereas a high-flux dialyser had no effect. The three dialyser types used in this study are made from the same material and have the same surface area and membrane thickness. The better performance of the super-flux dialyser can thus not be attributed to a larger surface area or different adsorptive characteristics of the membrane material. The three dialysers only differ with respect to pore size and ratio of open pores, resulting in a different ultrafiltration coefficient and clearance of middle-molecule substances.

The influx of Hcy into plasma is ~55 μmol/h and is similar in patients with renal failure and in healthy controls [15]. Plasma Hcy exists as a free and a protein-bound fraction, the latter with albumin as the principal carrier. In haemodialysis patients, 75% of Hcy is protein bound, a somewhat higher percentage than in the general population [16]. Consequently, diffusive clearance of Hcy is limited, despite its low molecular weight [9]. The amounts of Hcy recovered from dialysate were, indeed, very low compared with the influx of Hcy into plasma. In addition, the dialytic Hcy removal and the Hcy reduction ratio were similar for the three dialysers. These results therefore do not support the notion that the Hcy-lowering effect of the super-flux membrane is related to an enhanced dialytic clearance of Hcy. Further, the dialytic albumin loss of ~5 g per super-flux dialysis did not result in a decrease in serum albumin levels and thus does not provide an explanation for the Hcy-lowering effect of the membrane.

Patients with renal failure have a markedly reduced plasma clearance of Hcy [15], but the mechanism behind this observation is debated. The enzymes required for Hcy metabolism are found mainly in hepatocytes and proximal tubular epithelial cells. It has been suggested that normal kidneys are the main site of Hcy clearance and metabolism and that the Hcy accumulation in renal disease results from a reduced clearance and metabolism of Hcy by the failing kidneys. A substantial renal uptake and metabolism of Hcy has been reported in animal experiments [17,18], but no net renal extraction of Hcy occurred in fasting healthy volunteers [19]. The hypothesis was thus expounded that retained uraemic toxins with inhibitory effects on Hcy metabolism are primarily responsible for the hyperhomocysteinaemia of renal failure. A delayed post-dialytic rise in Hcy levels has been observed, whereas plasma creatinine began to increase immediately [9,10], suggesting that dialysis reduced the uraemic impairment of Hcy metabolism. We provide evidence for the first time strongly supporting the premise of inhibition of Hcy metabolism by uraemic toxins, thus improving the understanding of the aetiology of hyperhomocysteinaemia in renal failure. Super-flux dialysers may be more efficient in removing middle-molecular weight retention products with inhibitory activity against relevant enzymes and thus increase plasma clearance of Hcy. The lower tHcy concentrations among patients treated by daily nocturnal dialysis compared with conventional dialysis [20] may also relate to this mechanism.

In conclusion, modifications of the dialysis regimen in order to improve clearance of middle molecules are likely to have modest beneficial effects on tHcy concentrations in chronic haemodialysis patients. Whether this will translate into a better cardiovascular outcome should be the subject of further investigation.

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Conflict of interest statement. None declared.

References

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