Long-term use of vitamin E-coated polysulfone membrane reduces oxidative stress markers in haemodialysis patients

Hisanori Morimoto, Kazushi Nakao, Kousuke Fukuoka, Ai Sarai, Ai Yano, Takashi Kihara, Shinji Fukuda, Jun Wada and Hirofumi Makino

Department of Medicine and Clinical Science Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama and Innoshima General Hospital, Hiroshima, Japan

Abstract

Background. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase and an independent predictor of overall mortality and cardiovascular outcome in haemodialysis (HD) patients. In the present study, we compared the effects of a vitamin E-coated polysulfone membrane (PSE) and a non-vitamin E-coated polysulfone membrane (PS) on oxidative stress markers such as ADMA.

Methods. Thirty-one HD patients were enrolled to this investigation. They were allocated into two groups: in the PSE group (n = 16), PSE was used for 6 months, followed by PS for an additional 12 months; in the PS group (n = 15), PS was used for the entire observation period. Plasma ADMA, oxidized low density lipoprotein (Ox-LDL) and malondialdehyde LDL (MDA-LDL) levels were measured at baseline, 3, 6, 12 and 18 months. Plasma ADMA in peritoneal dialysis (PD) patients and in healthy individuals was also measured.

Results. Predialysis concentrations of ADMA (0.72 ± 0.13 nmol/ml) were significantly higher in the HD group than in both PD patients (0.63 ± 0.10 nmol/ml, P < 0.01) and healthy individuals (0.44 ± 0.01 nmol/ml, P < 0.0001). Treatment with PSE for 6 months significantly reduced predialysis levels of ADMA (0.54 ± 0.09 nmol/ml) compared with baseline (0.74 ± 0.12 nmol/ml; P < 0.01). Predialysis levels of Ox-LDL and MDA-LDL after 6 months therapy with PSE were also significantly lower than baseline values. Treatment with PS subsequent to treatment with PSE again increased ADMA, Ox-LDL and MDA-LDL back to baseline levels. In the PS group, ADMA, Ox-LDL and MDA-LDL levels remained unchanged during the entire treatment period of 18 months.

Conclusions. We confirmed that use of PSE reduced ADMA that had accumulated in HD patients. This finding indicates that PSE exerts anti-oxidant activity. A randomized controlled study will be required to determine whether PSE prevents cardiovascular diseases and other dialysis-related complications by reducing oxidative stress.

Keywords: ADMA; haemodialysis; oxidative stress; polysulfone; vitamin E-coated

Introduction

Cardiovascular disease is a major complication in patients with end-stage renal disease (ESRD) and is the single leading cause of death in haemodialysis (HD) patients. Therefore, to evaluate the effectiveness of therapeutic interventions or to predict patient outcome, it is necessary to measure various atherogenic factors. Among the proposed causes of atherogenesis, oxidative stress during HD is regarded as one of the critical determinants [1]. The contact of blood with the artificial dialysis membranes during HD sessions results in production of reactive oxygen species (ROS) by leukocytes [2]. Furthermore, it has been reported that serum antioxidant activity levels are significantly lower in chronic renal failure. In HD patients, oxidative stress is dominant because the balance between oxidant production and antioxidant activity shifts in favour of the former.

Nitric oxide (NO), which is generated from L-arginine by NO synthases (NOS), is an important substance in the regulation of vascular tone, blood pressure, cell-to-cell contact, and proliferation [3]. Therefore, a decreased bioavailability of NO leads to cell adhesion, proliferation and vasoconstriction, as well as acceleration of arteriosclerotic lesions. Asymmetric dimethylarginine (ADMA) is an...
endogenous inhibitor of endothelial nitric oxide synthase. Vance and colleagues [4,5] were the first to report that ADMA and its biologically inactive stereoisomer symmetric dimethylarginine (SDMA) are elevated in patients with ESRD [4,5]. In healthy subjects, ADMA is primarily eliminated by renal excretion and partly metabolized by dimethylarginine dimethylamino hydrolase (DDAH). The activity of DDAH decreases during oxidative stress conditions [1]. It was previously reported that ADMA levels were positively correlated with age, mean arterial pressure, glucose intolerance and carotid intima-media thickness in human subjects who showed no signs of coronary or peripheral arterial diseases [6]. Increases in carotid intima-media thickness are associated with an increased incidence of major cardiovascular events. In patients with ESRD, accumulation of ADMA may contribute to hypertension and immune dysfunction [4]. Furthermore, Zoccali et al. [3] found that ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome in HD patients.

Vitamin E is a fat-soluble antioxidant that plays a central role in reducing lipid peroxidation and in inhibiting the generation of ROS. We previously reported that long-term HD treatment with vitamin E-coated regenerated cellulose membranes reduced oxidative stress during HD sessions, as measured by malondialdehyde (MDA), advanced glycation end product (AGE) and 8-hydroxydeoxyguanosine levels [7]. In the present study, we evaluated whether vitamin E-coated polysulfone membranes (PSE), which may be more biocompatible, reduce oxidative stress as assessed by measuring ADMA, oxidized low density lipoprotein (Ox-LDL) and malondialdehyde low density lipoprotein (MDA-LDL).

Subjects and methods

Patients

Thirty-one patients undergoing chronic HD at the dialysis centre of Innoshima General Hospital were randomly selected. The study was approved by the ethical committee at the Innoshima General Hospital. Written informed consent was obtained from all patients. The mean age was 69.2±11.2 years (range, 44–88 years; 15 males and 16 females) and the duration of dialysis varied from 7 to 206 months (75.9±60.9 months). They underwent HD with a non-vitamin E-coated polysulfone membrane (surface area from 1.3 to 1.9 m²; Fresenius-Kawasumi, Tokyo, Japan); after use of PSE for 6 months, the membranes were switched to vitamin E-coated polysulfone membranes (surface area from 1.2 to 1.8 m²; Asahi Kasei Medical, Tokyo, Japan); after use of PSE for 6 months, the membranes were changed to PS again followed by 12 months of observation. In the PS group, PS was continued for the entire observation period. There were no statistical differences in the age, duration of dialysis, sex, percentage of diabetes patients, blood flow, surface area of dialysis membrane, dosages of recombinant erythropoietin administration or other biochemical parameters between two groups at baseline (Table 2).

Table 1. Patient backgrounds

<table>
<thead>
<tr>
<th></th>
<th>PSE</th>
<th>PS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>8:8</td>
<td>7:8</td>
<td></td>
</tr>
<tr>
<td>DM:non DM</td>
<td>7:9</td>
<td>7:8</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.4±10.6</td>
<td>69.0±12.2</td>
<td></td>
</tr>
<tr>
<td>Duration of dialysis</td>
<td>77.8±65.1</td>
<td>73.9±58.3</td>
<td></td>
</tr>
<tr>
<td>Surface area of dialysis membrane (m²)</td>
<td>1.58±0.28</td>
<td>1.52±0.27</td>
<td></td>
</tr>
<tr>
<td>Blood flow (ml/min)</td>
<td>261.9±18.0</td>
<td>262.0±17.8</td>
<td></td>
</tr>
</tbody>
</table>

Data are means±SD. NS, not significant.

Table 2. Biochemical parameters in haemodialysis patients at baseline

<table>
<thead>
<tr>
<th></th>
<th>PSE</th>
<th>PS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.9±0.7</td>
<td>9.6±1.5</td>
<td></td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>29.8±1.8</td>
<td>29.5±4.7</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.1±0.5</td>
<td>6.4±0.5</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.4±0.4</td>
<td>3.4±0.3</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>158.6±41.6</td>
<td>156.3±28.7</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>44.3±11.7</td>
<td>39.4±11.3</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>90.2±33.7</td>
<td>89.9±22.4</td>
<td></td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>58.2±21.3</td>
<td>56.3±11.9</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>9.3±2.3</td>
<td>8.3±2.1</td>
<td></td>
</tr>
<tr>
<td>β2-microglobulin (mg/l)</td>
<td>32.8±4.8</td>
<td>33.3±6.2</td>
<td></td>
</tr>
<tr>
<td>Kt/V urea</td>
<td>1.62±0.29</td>
<td>1.57±0.32</td>
<td></td>
</tr>
<tr>
<td>PCR (g/kg/day)</td>
<td>0.92±0.19</td>
<td>0.79±0.17</td>
<td></td>
</tr>
<tr>
<td>%CGR (%)</td>
<td>92.3±22.2</td>
<td>84.5±20.7</td>
<td></td>
</tr>
<tr>
<td>Hs-CRP (ng/ml)</td>
<td>2698±2110</td>
<td>4689±4485</td>
<td></td>
</tr>
<tr>
<td>EPO doses (U/week)</td>
<td>5464±1512</td>
<td>3363±2934</td>
<td></td>
</tr>
</tbody>
</table>

Data are means±SD. NS, not significant.

Blood sampling

Blood samples were drawn from arteriovenous fistulas prior to the first HD session of the week. Blood samples were collected at baseline, 3, 6, 12 and 18 months. At baseline, blood samples were obtained before the dialysis session and 5 min before the end of HD. Samples were immediately mixed with potassium-ethylenediaminetetraacetic acid and
centrifuged at 1670 × g for 5 min, and plasma was frozen at −20°C until assay. Serum samples were also stored. We evaluated changes in SDMA and oxidative stress markers, such as ADMA, Ox-LDL and MDA-LDL. In addition, we also evaluated the dialyser clearance of ADMA and SDMA. The dialyser clearance was calculated as follows: (predialysate plasma concentration – postdialysate plasma concentration) × blood flow (200 ml/min)/predialysate plasma concentration. Blood samples for the measurement of dialyser clearance were obtained at 1 h after the beginning of HD. Blood samples were collected after blood flow had been reduced to 200 ml/min. Five patients in the PSE group were recruited for the evaluation of dialyser clearance of ADMA and SDMA at baseline.

Assays

Plasma ADMA and SDMA concentrations were measured by high-performance liquid chromatography using precolumn derivatization with o-phthalaldehyde (OPA) [10]. In brief, the sera were deproteinized with ethanol, OPA was added to supernatant, and this was injected into the HPLC system. For measuring the concentrations of Ox-LDL and MDA-LDL, we first obtained LDL fractions by sequential ultracentrifugation. Using this fraction, the concentrations of Ox-LDL were measured with sandwich ELISA using anti Ox-LDL monoclonal antibody (FOH1a/DLH3) and anti-human apolipoprotein B antibody according to the methods of Itabe et al. [11]. The concentrations of MDA-LDL were determined from the formation of thiobarbituric acid reactive substances according to the method of Yagi [12]. Briefly, lipids and proteins were precipitated using 10% phosphotungstic acid and N/12 sulphuric acid. The sediment was resuspended in distilled water followed by the addition of thiobarbituric acid. The reaction mixture was heated at 95°C for 60 min; thiobarbituric acid-reacting substances were extracted with butanol. After centrifugation, the butanol layer was taken for fluorometric measurement at 515 nm excitation and 550 nm emissions. Measurement of serum β2-microglobulin (β2m) was carried out by radioimmunoassay. The percentage of reduction in serum β2m concentration was calculated as follows: [(serum concentration predialysis – serum concentration postdialysis)/serum concentration predialysis] × 100%.

Statistical analysis

Data are presented as means ± SD. Differences between groups were examined for statistical significance using unpaired and paired t-tests. Statistical significance was set at P < 0.05.

Results

Predialysis ADMA levels were elevated in HD patients

We first investigated plasma ADMA concentrations in healthy controls (n = 20, age 65.4 ± 6.3 years, male:female = 10:10), peritoneal dialysis (PD) patients (n = 43, age 66.6 ± 11.2 years, male:female = 20:23), and in HD patients (n = 31, age 69.2 ± 11.2 years, male:female = 15:16). There were no differences in age or sex between the control, PD and HD groups. Residual renal function of PD patients was significantly higher than in HD patients (PD, 523 ± 446 ml; HD, 110 ± 101 ml of urine/24 h; P < 0.01). Duration of dialysis in the HD group (75.9 ± 60.9 months) was longer than in the PD group (33.7 ± 21.3 months). Predialysis concentrations of ADMA in the HD group (0.72 ± 0.13 nmol/ml) were significantly higher than those of PD (0.63 ± 0.10 nmol/ml, P < 0.01) and healthy control groups (0.44 ± 0.01 nmol/ml, P < 0.0001) (Figure 1).

Fig. 1. Plasma levels of ADMA in patients with ESRD. Shown are comparisons of ADMA levels among healthy individuals and ESRD patients treated with PD and HD. Plasma ADMA concentrations in ESRD patients were significantly higher than in healthy individuals. Furthermore, ADMA levels were significantly elevated in HD patients compared with PD patients.

Dialyser clearance of ADMA and SDMA using PSE and PS

Five patients in the PSE group were enrolled to evaluate dialyser clearance of ADMA and SDMA at baseline. There was no statistical difference in dialyser clearance of ADMA between PSE and PS (160.1 ± 5.8 ml/min) and PS (163.4 ± 5.4 ml/min). Similarly, the dialyser clearance of SDMA was not different between PSE (161.7 ± 1.8 ml/min) and PS (164.5 ± 2.0 ml/min).

ADMA concentrations were reduced during single sessions of haemodialysis with PSE and PS

The basal or predialysis ADMA, Ox-LDL and MDA-LDL concentrations were not different between the PSE and PS groups. The post-dialysis ADMA concentrations were decreased by ∼40% in both groups (PSE group, from 0.74 ± 0.03 to 0.41 ± 0.02 nmol/ml; PS group, from 0.69 ± 0.04 to 0.41 ± 0.02 nmol/ml) (Figure 2A). After a single HD session, plasma Ox-LDL levels were increased by 50% in both groups (PSE, from 1.73 ± 0.13 to 2.61 ± 0.19 ng/μg LDL protein; PS, from 1.88 ± 0.11 to 2.69 ± 0.19 ng/μg LDL protein) (Figure 2B). Plasma MDA-LDL did
not change during the HD session in either group (PSE, from 4.47 ± 0.42 to 4.41 ± 0.45 nmol/mg LDL protein; PS, from 4.60 ± 0.22 to 4.62 ± 0.33 nmol/mg LDL protein) (Figure 2C).

**Long-term use of PSE reduced predialysis levels of ADMA, Ox-LDL and MDA-LDL**

The long-term effects of HD using PSE and PS on oxidative stress markers were prospectively investigated (Figure 3A, C and D). We also evaluated whether plasma SDMA, and elimination of SDMA solely depends on renal and dialytic excretion and not on DDAH activities (Figure 3B). In the PSE group, ADMA concentrations were significantly decreased at 3 (0.57 ± 0.08 nmol/ml) and 6 months (0.54 ± 0.09 nmol/ml) compared with baseline levels (0.74 ± 0.12 nmol/ml; P < 0.01). ADMA levels at 6 months were significantly lower than in the PS group (0.62 ± 0.06 nmol/ml; P < 0.05). In contrast, ADMA levels increased at 18 months (0.66 ± 0.12 nmol/ml; P < 0.01, vs 6 months) after the usage of PS. ADMA levels in the PS group did not change during the observation period. SDMA concentrations in the PSE (2.89 ± 0.55 nmol/ml) and PS groups (2.89 ± 1.07 nmol/ml) at baseline were about 4-fold higher compared with ADMA concentrations at baseline. During the observation period, SDMA levels in both groups did not significantly change. Ox-LDL
concentrations in HD patients treated with PS and PSE were also evaluated. At 6 months, Ox-LDL levels decreased significantly in the PSE group (1.31 ± 0.49 ng/µg LDL protein) compared with basal level at month 0 (1.73 ± 0.47 ng/µg LDL protein; P < 0.01). After treatment with PS, Ox-LDL levels increased significantly at 18 months (1.77 ± 0.53 ng/µg LDL protein) compared with 6 months (P < 0.01). Ox-LDL levels at 6 months were significantly lower than in the PS group (1.92 ± 0.84 ng/µg LDL protein; P < 0.05). Ox-LDL levels in the PS group did not change during the observation period. Measurements of plasma MDA-LDL concentrations indicated that 3 months of treatment with both membranes did not change MDA-LDL levels. In the PSE group, MDA-LDL levels were significantly reduced at 6 months (3.16 ± 0.99 nmol/mg LDL protein; P < 0.01) compared with month 0 (4.47 ± 1.55 nmol/mg LDL protein) and 3 months (4.51 ± 1.83 nmol/mg LDL protein). MDA-LDL levels in the PSE group at 6 months were significantly lower than in the PS group (4.64 ± 0.96 nmol/mg LDL protein; P < 0.01). After treatment with PS, MDA-LDL levels returned to baseline levels at 12 and 18 months. Although haemoglobin levels remained stable during the observation periods in both groups, the dosages of erythropoietin required to maintain haemoglobin levels were significantly reduced at 6 months in the PSE group. In contrast, the required erythropoietin dosages significantly increased after reverting to PS. K\textsubscript{t}/V for urea, PCR and %CGR did not significantly change during the observation periods (Table 3).

**Discussion**

Oxidative stress is known to contribute to the development of atherosclerotic diseases in HD patients. In addition, oxidative stress is present in chronic renal failure patients, and particularly in HD patients. In these HD patients, there are frequent opportunities for direct contact between leukocytes and a dialysis membrane. Since a dialysis membrane is an artificial biomaterial, the leukocytes and complements are activated to produce a variety of reactive substances, including superoxide, hydrogen peroxide and hypochlorous acid [13]. The use of a bioincompatible dialysis system results in a dramatic increase in the production of ROS, thus reducing antioxidant defence mechanisms. Recently, the biocompatibility of synthetic membranes has been markedly improved. However, many issues related to prominent oxidative stress during HD remain largely unresolved.

Vitamin E acts as a powerful radical scavenger that protects plasma lipids and cell membranes from peroxidative events. Previously, we found that vitamin E-coated regenerated cellulose membranes were effective in protecting HD patients against damage caused by oxidative stress to lipids, proteins and nucleic acids during long-term HD treatment [7]. Based on these findings, and since PSE is more biocompatible and has a higher reduction rate of low molecular weight proteins compared with the regenerated cellulose membranes, we evaluated whether long-term use of PSE reduces oxidative damage. We investigated the polysulfone membrane, wherein the presence of endotoxin in dialysate may promote oxidant production. However, we used the reverse osmosis system and endotoxin cut filters to provide endotoxin-free dialysate and, as a result, endotoxin levels in the dialysate were below detection limits. Thus, endotoxin from dialysate back filtration was considered to have minimal effect on HD patients in the present study.
Endothelial vasodilator dysfunction has been implicated as a key event in the pathogenesis of arteriosclerosis. The endothelium plays a crucial role in the maintenance of vascular tone by releasing vasoactive mediators, such as NO, which is formed by NOS. Other roles of NO include inhibition of platelet aggregation, monocyte adhesion and of smooth muscle cell proliferation. It is believed that endothelial dysfunction caused by reduced NO production is associated with the development of arteriosclerosis, and this results in ischaemic manifestations such as acute coronary syndrome and stroke [14]. The synthesis of NO is selectively inhibited by analogues of L-arginine in which the guanidine nitrogen is substituted, such as N-monomethyl-L-arginine (NMA) and ADMA. ADMA concentrations, which are 10-fold higher than NMA, may represent a more important endogenous inhibitor of NOS than NMA. We therefore decided to evaluate ADMA as a major oxidant marker in HD patients treated with PSE.

Figure 4 shows the metabolic pathways for the generation and degradation of ADMA. L-arginine residues within proteins are methylated by the protein arginine methyltransferase (PRMT) type 1, which utilizes S-adenosylmethionine as a methyl group donor [1]. There are two types of PRMT: ADMA and NMA are formed by PRMT type 1, whereas PRMT type 2 forms SDMA, which has no inhibitory effect on NOS [15]. Free NMA and ADMA are released during proteolytic breakdown. ADMA is excreted in part into the urine, but is mainly metabolized into L-citrullin and dimethylamine by the action of DDAH. In contrast, SDMA is secreted into the urine and is eliminated by dialysis; however, SDMA was not enzymatically metabolized by DDAH.

In the current study, plasma ADMA concentrations in ESRD patients were significantly higher than in healthy individuals (Figure 1); similar findings have already been reported [3]. This finding may be explained by two major mechanisms. First, the accumulation of ADMA may be due to deterioration of renal function [4], and secondly, high plasma ADMA levels may be caused by oxidative stress in patients with ESRD. In favour of the latter mechanism, oxidative stress causes high expression of PRMT and suppression of DDAH activity, which may contribute to the elevation of ADMA concentrations in patients with ESRD [1]. Studies measuring plasma levels of ADMA in HD and PD patients have reported conflicting findings. While Anderstam et al. [16] found that ADMA concentrations in HD patients were not different from PD patients, Kielstein et al. [17] reported that PD patients had lower ADMA levels. These disparities may be due to the considerable differences in residual renal function and dialytic clearance of peritoneum in the PD patients from the current investigation and reported studies.

Zoccali et al. [3] showed that plasma ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome in HD patients. Our finding that plasma ADMA concentrations were reduced after single HD sessions while using both membranes (see Figure 2A) suggests that ADMA, with a molecular weight of 202 Da, is efficiently eliminated by HD. Interestingly, we also showed a significant decrease in predialysis ADMA levels after long-term HD treatment with PSE (see Figure 3A). There was no significant difference in the dialyser clearance of ADMA between PSE and PS. We therefore suggest that the effect of ADMA removal during a single HD session does not contribute to the decrease in plasma ADMA levels following long-term use of PSE. Saran et al. [18] reported that oral administration of high dose vitamin E for 8 weeks reduced plasma ADMA levels in chronic kidney disease patients, which gave rise to our hypothesis that vitamin E-coated polysulfone membranes may reduce the local production of ROS and diminish oxidative stress.

In a previous publication, we suggested that vitamin E-coated regenerated cellulose membranes may protect HD patients against damage caused by oxidative stress [7]. We currently observed significant reductions in Ox-LDL and MDA-LDL levels while using PSE after long-term HD treatment (see Figure 3C and D). Moreover, we evaluated predialysis plasma SDMA, which was not metabolized by DDAH (Figure 3B). Unlike ADMA, SDMA levels did not change in either group during the observation periods, supporting the hypothesis that vitamin E-coated polysulfone membranes reduce oxidative stress and increase DDAH activity to thereby lower ADMA levels. On the basis of our available data, long-term HD treatment using PSE is putatively associated with protection against oxidative stress. It is theoretically possible that improvements in both PRMT expression and DDAH activities, caused by amelioration of long-term oxidative damage using PSE, may play a crucial role in reducing plasma ADMA concentrations. Previously, the use of statins effectively lowered LDL-cholesterol.
without affecting ADMA levels [19], which supports the idea that anti-oxidant activity may be a major mechanism for ADMA reduction during dialysis with PSE.

\( \beta_2m \) is a precursor protein of dialysis-related amyloidosis, and an important marker of low molecular weight protein accumulation in chronic HD patients. Reduction rates of \( \beta_2m \) during single HD sessions have been reported to be higher with polysulfone membranes than with regenerated cellulose membranes. The higher filtration of \( \beta_2m \) through polysulfone membranes is due to their large pore size. Since we had been using the polysulfone membrane in all enrolled patients before this study, the change in predialysis \( \beta_2m \) levels was not seen during the observation periods (data not shown). In previous reports, there were no differences in serum \( \beta_2m \) concentrations between dialysis patients with and without amyloidosis, even though retention of \( \beta_2m \) is thought to be an essential factor for the onset of amyloidosis. An additional causative factor may be the accumulation of AGE. The generation of AGE is enhanced by increased oxidative stress, which is associated with uraemia. AGE-modified \( \beta_2m \) is responsible for macrophage chemotaxis, activation and cytokine release, which is implicated in the process of bone and joint destruction during dialysis-related amyloidosis. In the current investigation, we showed that PSE may exert two beneficial actions during HD therapy. One of these is the efficient removal of a number of substances from middle to large molecules, and the second is the capacity for reducing oxidative stress. Given these findings, we suggest that vitamin E-coated polysulfone membranes may prevent dialysis-related amyloidosis.

Although haemoglobin levels did not change during the observation periods, the erythropoietin doses required to maintain haemoglobin were significantly decreased during PSE at 6 months. HD treatment with vitamin E-coated cellulose membrane may improve the rheology of circulating red blood cells, and reduce the requirement of erythropoietin doses in HD patients. In addition, reductions in oxidant stress may contribute to a sparing effect on exogenous erythropoietin administration. It was recently reported that erythropoietin decreased DDAH activity and increased ADMA levels, which may explain why erythropoietin increases hypertension and cardiovascular risk [20]. Thus, the reduced doses of erythropoietin may partly explain the reduced plasma levels of ADMA.

In conclusion, the present study demonstrated that biocompatible polysulfone membranes coated with vitamin E reduce oxidative stress. Long-term randomized control studies will be required to determine whether prognosis is improved and whether chronic vascular complications are reduced by long-term use of PSE.

**Acknowledgements.** The authors gratefully acknowledge the excellent technical support of H. Nishi, R. Kondo and the staff of the Dialysis Center at Innoshima General Hospital.

**References**


**Conflict of interest statement.** None declared.


Received for publication: 2.2.05
Accepted in revised form: 26.7.05