Vitamin E–bonded hemodialyzer improves atherosclerosis associated with a rheological improvement of circulating red blood cells

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Vitamin E–bonded hemodialyzer improves atherosclerosis associated with a rheological improvement of circulating red blood cells.

Background. Vitamin E–bonded hemodialyzer is known to improve oxidative stress in patients with hemodialysis. However, there is little information available as to whether or not this membrane clinically improves atherosclerosis. Furthermore, it remains unknown whether there is any effect of the membrane on rheology of circulating red blood cells.

Method. We conducted a randomized, open-labeled, prospective control study (N = 34) for 1 year to investigate the effect of vitamin E–bonded cellulose membrane dialyzer (EE) (N = 17) on carotid atherosclerotic changes [intima-media thickness (IMT) of carotid arteries] and the viscosity, percentage of dysmorphism (%DMR) of red blood cells (RBCs) and their distribution width-standard deviation (RDW-SD), in comparison with cellulose membrane (SU) (N = 17) identical to EE without vitamin E–bonded membrane. Erythropoietin (EPO) dose used for the treatment of uremic anemia was also calculated.

Results. The IMT significantly decreased in the EE group, while in the SU group the IMT significantly increased. The viscosity of RBCs in hemodialysis patients (4.70 ± 0.45 cP) was greater than that in healthy individuals (3.73 ± 0.15 cP). EE significantly improved the viscosity (from 4.84 ± 0.41 cP to 4.51 ± 0.54 cP, P < 0.01), %DMR (from 2.29 ± 2.17% to 1.90 ± 1.49%, P < 0.01), and RDW-SD (from 54.4 ± 7.6 fL to 49.3 ± 5.9 fL, P < 0.01). On the contrary, these parameters all worsened in the SU group. EPO dose needed for the treatment of anemia was significantly (P < 0.05) reduced from 5383 ± 2655 U/week to 4235 ± 3103 U/week in the EE group. During these period, mean blood pressure, Kt/V urea, and serum β2-microglobulin were not changed between the two groups.

Conclusion. These findings suggest that vitamin E–bonded hemodialyzer is very useful for improving atherosclerosis from a clinical point of view. As one of the underlying mechanisms, as well as antioxidant effects, we want to address an important role of the improvement of rheology of circulating RBCs, which may also help to reduce the requirement of EPO dose in the treatment of anemia of ESRD patients.

Key words: vitamin E, hemodialysis, atherosclerosis, rheology, intima-media thickness, viscosity, erythropoetin.

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Atherosclerosis has much impact on mortality and morbidity of patients undergoing hemodialysis. Important determinants of the outcome in dialysis patients are known to be cardiovascular disorders. Oxidative stress is known to contribute to the development and progression of atherosclerotic cardiovascular complications in hemodialysis patients [1]. Antioxidants such as vitamin E are reported to reduce the level of serum reactive-oxygen species (ROS) or oxidized low-density lipoproteins (Ox-LDLs) in patients with hemodialysis [2]. Although it still remains unknown whether or not hemodialysis per se affects the progression of the complications, the patients undergoing long-term hemodialysis tend to have more serious cardiovascular complications. This may be due to bioincompatibility between blood and dialysis membranes. The bioincompatibility contributes to the oxidative stress, which leads to atherosclerosis [3]. Mechanical contact of leukocytes with a dialysis membrane induces the activation of ROS during hemodialysis [4]. In this regard, the development of a new biocompatible membrane is necessary for reducing oxidative stress by lowering the circulating ROS.

Recently, vitamin E–bonded hemodialyzers have been developed, which is known to have many favorable effects (i.e., the reduction of interleukin-6 release from monocytes [5] or improvement of endothelial dysfunction [6] during hemodialysis). However, there is little information available regarding to what extent they clinically improve atherosclerosis, although it has been recently reported that this membrane reduces a percentage increase in aortic calcification [7]. A significant increase in the intima-media thickness (IMT) of the carotid artery, a good indicator of atherosclerosis, is reported in dialysis patients, as compared with age-matched healthy subjects [8]. In the present study, we wanted to see how IMT is affected by vitamin E–bonded hemodialyzers. Furthermore, as one of the possibilities underlying mechanism(s) in which vitamin E–bonded dialyzer may exert its favorable effects on atherosclerosis, we consider that
this membrane affects the rheology of peripheral circulation. Vitamin E–bonded membrane is known to change the oxidation of red blood cell (RBC) membranes [9]. Thus, in terms of rheology, we have focused on viscosity, dysmorphism (DMR) of RBCs, and the RBC distribution width–standard deviation (RDW-SD) obtained from the size distribution curve of RBCs since the viscosity of RBCs in the circulation may be related to injuries of vascular endothelial cells, thus inducing the activation of platelets, which then leads to thrombosis. In addition, there is a possibility that an improvement of the rheology may lead to affect erythropoietin (EPO) dose used during hemodialysis.

The aim of our present study is to investigate whether vitamin E–bonded membrane retards the increase in IMT and to investigate the relationship between its viscosity, the percentage of DMR, and RDW-SD of RBCs as well as EPO dose used for the treatment of uremic anemia. To conduct these studies, we compared two membranes, vitamin E–bonded and controlled membrane, which is identical except the surface modified with vitamin E, in a randomized, open-labeled, prospective study for 1 year.

METHODS

Patients

Thirty-four clinically stable patients in our hospital were randomly selected. The patients included 19 males and 15 females with a mean age of 62 ± 12 years (range, 36 to 83 years). The mean time on hemodialysis treatment was 4.4 ± 1.1 years, ranging from 0.9 to 8.9 years. The causes of ESRD included chronic glomerulonephritis (N = 26) and diabetes (N = 8). No patient had hematologic disorders such as lymphoma, myeloma, or other related diseases. These patients were divided into two groups, according to dialysis membrane, a vitamin E–bonded membrane (CL-EE; Terumo Co., Ltd., Tokyo, Japan) or control membrane, which is identical to the CL-EE except that membrane was not bonded to vitamin E (CL-SU; Terumo Co., Ltd., Tokyo). There was no statistical difference in the age and the mean hemodialysis treatment time between each group (Table 1). Regarding concurrent medications, eicosapentanoic acid was given in six of 17 patients in the EE group and seven of 17 patients in the SU group, although statin was not used. Prior to the study, there were three kinds of dialyzer used, regenerated cellulose membrane, polysulfone, and polymethylmethacrylate. However, there was statistically no difference between the groups. Both groups were prospectively followed up for 1 year. All the patients were informed of the purpose of the study and gave their informed consent. The protocol for this study was approved by the Institutional Review Board.

Table 1. Comparison of patient characteristics in vitamin E–bonded cellulose membrane dialyzer (EE) and a cellulose membrane without vitamin E–bonded membrane (SU)

<table>
<thead>
<tr>
<th>Group</th>
<th>EE</th>
<th>SU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Age years</td>
<td>60.9 ± 13.0</td>
<td>63.2 ± 10.7</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>10/7</td>
<td>9/8</td>
</tr>
<tr>
<td>Duration of hemodialysis years</td>
<td>4.4 ± 2.6</td>
<td>4.4 ± 2.7</td>
</tr>
<tr>
<td>Qb mL</td>
<td>182 ± 16</td>
<td>186 ± 24</td>
</tr>
<tr>
<td>Kt/V urea</td>
<td>1.31 ± 0.13</td>
<td>1.28 ± 0.13</td>
</tr>
<tr>
<td>Serum β2-microglobulin</td>
<td>37.1 ± 6.55</td>
<td>38.2 ± 7.92</td>
</tr>
<tr>
<td>PCR g/kg/day</td>
<td>1.08 ± 0.03</td>
<td>1.09 ± 0.02</td>
</tr>
<tr>
<td>MBP mm Hg</td>
<td>110 ± 2</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>BMI</td>
<td>21.2 ± 3.6</td>
<td>21.7 ± 3.5</td>
</tr>
<tr>
<td>TC mg/dL</td>
<td>150 ± 37</td>
<td>153 ± 32</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>120 ± 78</td>
<td>123 ± 71</td>
</tr>
<tr>
<td>HDL mg/dL</td>
<td>45 ± 17</td>
<td>44 ± 14</td>
</tr>
<tr>
<td>Alb g/dL</td>
<td>3.9 ± 0.4</td>
<td>4.0 ± 0.3</td>
</tr>
</tbody>
</table>

 Abbreviations are: PCR, protein catabolic rate; MBP, mean blood pressure; NS, not statistically significant; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoproteins; Alb, albumin.

Hemodialysis-related factors

In vitro data show that solutes clearance in both dialyzers (1.5 m²surface area) are 187 mL/min versus 185 mL/min for urea, 172 mL/min versus 168 mL/min for creatinine, and 91 mL/min versus 77 mL/min for vitamin B₁₂ in the CL-EE and CL-SU, respectively. All the patients used bicarbonate dialysate with a flow rate of 500 mL/min. Blood flow rate during hemodialysis (Qb) was 3.5 mL/kg body weight. All these patients underwent 4-hour hemodialysis three times a week. Neither bacteria nor pyrogen was detected in the dialysate prepared from water obtained by reverse osmosis. No patient re-used the dialyzer membrane, following the standard practice in Japan. Endotoxin concentration in the dialysate was below 5 EU/L by routine analysis with Limulus Amebocyte Lysate assay (Wako Junyaku Endotoxin Measurement Kit, Tokyo, Japan). Efficacy of hemodialysis was assessed by the calculation of Kt/V according to the equation of Daugirdas [10]. Under the same conditions as described above, all patients were followed up for 12 months. Blood samples were drawn from the arterial site of arteriovenous fistula at the start and at the end of dialysis session at a 2-day interval. Serum was transferred into plastic tubes and stored at −80°C until its measure-ment. Only in the case of the measurement of RBC viscosity described below, the assay was performed within 24 hours and no stored sample was used.

Evaluation of atherosclerotic changes

Both carotid arteries were examined using a 7.5 MHz linear array transducer with high-resolution B-mode echography (Aloka, Tokyo, Japan). This device can measure the arterial wall thickness by 0.1 mm. The carotid arteries were examined bilaterally in the areas of the common carotid artery (1 cm proximal to the dilatation...
of the carotid bulb), the carotid bifurcation (1 cm proximal to the flow divider), and the internal carotid artery (1 cm distal to the flow divider) according to the method of Burke et al [11]. The IMT was defined as the distance between the leading edge of the lumen-intima echo of the near wall and the leading edge of the media-adventitia echo. The measurements of IMT were made blindly by two carefully trained sonographers. In a separate series of IMT measurements done in another patients, the interobserver coefficient of variation was 8%. To enhance the reproducibility of measurements, standardized interrogation angles were used according to the recommendations described previously [11]. The maximum thickness of the wall was recorded. The existence of plaque was also recorded. In cases where it was hard to tell the difference between plaques and thickened carotid walls, except by being judged as apparent plaques, the plaque was defined as the IMT of more than 1.5 mm. The prevalence of plaques was recorded, but the size was not.

The predialysis mean blood pressure was determined as mean blood pressure = diastolic + (systolic-diastolic)/3.

Analysis of viscosity of RBCs

To measure the viscosity of RBCs, we used cone plate type viscometer (Viscomic ED, Tokyo Keiki, Tokyo, Japan). RBCs obtained after the removal of plasma and buffy coat from heparinized blood were washed three times with 0.9% NaCl solutions, and adjusted in 55% hematocrit. According to the manufacturers’ instruction, 1.1 mL of RBC solution was added to viscometer at 37°C and viscosity was measured as a unit of cP (dyne • sec/cm²) 2 minutes after the incubation. Preliminary studies showed that the incubation time required was 2 minutes. Study of the reproducibility of measurements showed variable values within 8% of cP. To find how oxidative stress would affect cP, we studied the change of viscosity shown after an addition of 1 mmol/L CuSO4 solution.

Analysis of DMR of RBCs

Using a scanning electron microscopy, RBCs fixed with 2% glutaraldehyde were observed at a magnification of ×2000. Representative DMR of RBCs obtained by hemodialysis patients is shown in Figure 1. A percentage of any DMR of RBCs was measured according to the method of Calzavara et al [12] and compared between the two groups.

Analysis of RBC size distribution curve

RDW-SD of histogram for RBCs distribution curve, shown as a unit of fL, shows a degree of anisocytosis, which means a width of RBC distribution at a height of 20% from the bottom of the curve when the peak value of the distribution is 100%.

EPO dose for the treatment of ESRD anemia

All the patients had been given EPO intravenously as hematocrit was maintained at 30% to 33% with a level of ferritin between 100 ng/mL and 300 ng/mL. Every 2 weeks total counts of RBCs, hemoglobin, and hematocrit were measured and ferritin level was checked every 3 months. If ferritin level was below than 100 ng/mL, we administered iron intravenously, measuring the ferritin level every month. Under the conditions mentioned above, EPO requirements were adjusted every month.

Statistical analysis

All the data are expressed as mean ± SD. Differences between the two groups were analyzed by an unpaired and paired Student t test. Mann-Whitney U test was also used as necessary. The level of statistical significance was defined as P < 0.05. All the statistical analysis were performed with SAS/Windows (version 6.12).

RESULTS

All the 34 patients completed the study. Table 1 shows the patient characteristics. There was no difference in
Table 2. Effects of membrane on intima-media thickness (IMT) and erythropoietin (EPO)

<table>
<thead>
<tr>
<th></th>
<th>EE</th>
<th>SU</th>
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<tr>
<td>EPO U/week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5383 ± 2655</td>
<td>5162 ± 2514</td>
</tr>
<tr>
<td>After</td>
<td>4235 ± 3103</td>
<td>6618 ± 2190</td>
</tr>
<tr>
<td>Ferittin ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>136 ± 48</td>
<td>128 ± 46</td>
</tr>
<tr>
<td>After</td>
<td>130 ± 40</td>
<td>132 ± 41</td>
</tr>
<tr>
<td>Right IMT mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.93 ± 0.18</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>After</td>
<td>0.88 ± 0.22</td>
<td>0.99 ± 0.21</td>
</tr>
<tr>
<td>Prevalence of plaque %</td>
<td>35.3</td>
<td>29.4</td>
</tr>
<tr>
<td>Left IMT mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.97 ± 0.24</td>
<td>0.93 ± 0.19</td>
</tr>
<tr>
<td>After</td>
<td>0.87 ± 0.14</td>
<td>0.99 ± 0.25</td>
</tr>
</tbody>
</table>

Abbreviations are: EE, vitamin E–bonded cellulose membrane dialyzer; SU, cellular membrane without vitamin E–bonded membrane; NS, not statistically significant.

As shown in Table 2, Vitamin E–bonded dialyzer significantly ($P < 0.05$) decreased IMT, while that of the control group did not, but rather increased significantly. The prevalence of carotid plaque did not change in both the groups before and after this study.

Normal value of viscosity of RBCs was determined to be $3.73 ± 0.15$ cP ($N = 7$) using RBCs obtained from healthy individuals. Regarding the validity of viscosity measurement for oxidative stress, we confirmed that viscosity increased from 3.73 to 7.45 cP 3 hours after 1 mmol/L CuSO$_4$ addition as an oxidative stress to RBCs obtained from healthy individuals. RBCs viscosity in all these hemodialysis patients showed increased values, which means that hemodialysis patients are clearly under oxidative stress conditions. As shown in Figure 2, EE had been improving the viscosity from 4.84 ± 0.41 cP to 4.63 ± 0.36 cP after 6 months, and to 4.51 ± 0.54 cP at 1 year after this study, reaching to the statistically significant level ($P < 0.01$). On the other hand, SU rather aggravated the viscosity from 4.57 ± 0.48 cP to 4.72 ± 0.49 cP after 6 months, and to 4.90 ± 0.53 cP after 1 year ($P < 0.01$)

The percentage of DMR (%DMR) rate of RBCs showed the same trends. Normal value of %DMR was $0.77 ± 0.43$ ($N = 7$) using RBCs obtained from healthy individuals. As shown in Figure 3 a and b, RBCs from hemodialysis patients in the SU group showed numerous DMR, which were called largely echinocytes, compared to those in the EE group (Fig. 3 c and d). EE significantly decreased the %DMR from 2.29 ± 2.17% to 2.16 ± 1.79% after 6 months, and to 1.90 ± 1.49% ($P < 0.01$) after 1 year, while the SU group did not statistically improve the %DMR ($1.98 ± 1.44%$ before, $1.93 ± 1.45%$ after 6 months, and $1.88 ± 1.46%$ after 1 year).

Normal value of RDW-SD in health individuals was $45.1 ± 0.63$ fL ($N = 7$). As shown in Figure 4, RDW-SD likewise improved significantly ($P < 0.01$) in the EE group, from $54.4 ± 7.6$ fL to $49.1 ± 7.0$ fL after 6 months, and to $49.3 ± 5.9$ fL after 1 year. However, the SU group did not show any improvement ($55.3 ± 7.4$ fL before, $55.8 ± 6.9$ fL after 6 months, and $55.1 ± 6.2$ fL after 1 year).

As shown in Table 2, EPO dose used for the correction of anemia in ESRD was much reduced in the EE group,
from $5383 \pm 2655$ U/week to $4235 \pm 3103$ U/week ($P < 0.05$). In the SU group, on the contrary, EPO dose significantly increased from $5162 \pm 2514$ U/week to $6618 \pm 2190$ U/week ($P < 0.001$). Serum levels of ferritin were not significantly different.

**DISCUSSION**

We demonstrated that vitamin E–bonded dialyzer was clinically useful for the retardation of atherosclerosis shown in carotid IMT and to reduce EPO dose needed for the correction of anemia in ESRD. We also showed that these favorable effects may be ascribed to the improvement of rheology of peripheral circulation.

There are insufficient data available as to whether or not vitamin E–bonded dialyzer is indeed useful for atherosclerosis clinically found in ESRD patients, although there are many reports showing that this membrane clearly reduces an oxidative stress. Clinically, it has been already reported that EPO dose used for the correction of anemia decreased [13, 14]. Concerning the effect of the membrane on atherosclerosis, there is only a report that vitamin E–bonded dialyzer significantly reduces the percentage increase in aortic calcification index (ACI) evaluated by computed tomography (CT) [7]. Regarding repeatability of IMT measurements, there may be a number of potential limitations to our study. However, we added important evidence by showing that IMT was improved in the EE group 1 year later in the prospective controlled study, although the prevalence of plaque was not changed. Although blood pressure control is known to affect the progression of atherosclerosis, mean blood pressure was not changed between the two groups nor was it altered between before or after this study. Thus, this favorable effect appears to be due to the use of vitamin E–bonded dialyzer. Our results also confirmed the previous reports [13, 14] that EPO dose was reduced in patients using vitamin E–bonded dialyzer.

The vitamin E–modified dialyzer consists of a block polymer, which masks the hydroxyl groups on cellulose, an oleyl alcohol that inhibits platelet aggregation, and a vitamin E coating with antioxidant properties as re-
plasma levels of ox-LDL and malondialdehyde (MDA) [7, 9, 20]. Therefore, we did not measure these plasma levels of ox-LDL or MDA in the present study. Likewise it is well known that this membrane improves neutrophil function [20, 21] and reduces superoxide anion radicals [20]. Despite numerous reports regarding the favorable effects of vitamin E–bonded membrane on oxidative stress assessed by such plasma levels as ox-LDL or ROS, Dhondt et al [22] have recently reported that when comparing this membrane with polysulfone membrane regarding acute biocompatibility parameters, no superiority of this membrane versus the biocompatible standard polysulfone membrane could be shown.

Our interest regarding the underlying mechanisms in which the membrane exerts its favorable effect has been directed toward the rheology of circulating RBCs. There are no data available for the effect of vitamin E–bonded membrane on this field. Since RBCs show increased membrane lipid peroxidation, reduced membrane fluidity, and increased osmotic fragility [23–25] after hemodialysis, we tried to look at the changes of RBCs viscosity after the long-term use of the membrane. As expected the viscosity of RBCs was greater in hemodialysis patients, as compared with that in healthy subjects. After this study, cP increases in the SU group. Therefore, biocompatibility during hemodialysis is also very important factor to influence rheologic changes, which may lead to the progression of atherosclerosis. Unfortunately, we do not know the cP value at the induction of hemodialysis in individual cases. It remains unknown to what extent cP had been changing until the beginning of this study after hemodialysis induction. There might be a possibility that cP had increased in patients using bioincompatible membrane until the beginning of this study. At all events, we clearly demonstrated that vitamin E–bonded dialyzer had been decreasing the viscosity of RBCs as time goes by as well as other parameters, including %DMR or RDW-SD, while viscosity had been getting worse in the control SU group. To see the importance of rheologic changes of RBCs in reduction of IMT, we analyzed the correlation between the two. However, there was no correlation between any parameters of rheologic changes and IMT. This may be due to the fact that the change of IMT is too small. Our preliminary study shows that patients with chronic renal failure prior to the induction of hemodialysis have increased viscosity and DMR of RBCs. Therefore, these rheologic changes are not necessarily ascribed to the mechanical influences caused by hemodialysis. Regarding the mechanisms of how the changes of rheology could be related to atherosclerosis, there is little direct data available. We speculate as follows. The increased viscosity of RBCs in the circulation leads to injuries of endothelium, which then activates platelets or neutrophils. Indirectly, ROS or cytokines are released, or thrombus would be formed.
EPO dose used for the treatment was reduced in accord with an improvement of rheology mentioned above. In other words EPO dose used in the EE group significantly decreased, while EPO dose used in the SU group significantly increased. Although it is reported that this membrane is associated with less clotting in hemodialysis patients [14], the changed properties of RBCs membrane may contribute to the decreased viscosity or DMR, thus increasing erythrocyte survival [26]. Taken together, there is a possibility that an improvement of rheology of circulating RBCs, as well as antioxidant effects, improved carotid IMT.

CONCLUSION

We have shown that vitamin E–bonded hemodialyzer used for 1 year improved the increased IMT of carotid arteries, and reduced the EPO dose required for the treatment of ESRD anemia. The improved viscosity and DMR of RBCs clearly shown in the present study may contribute to these favorable effects through the changed properties of RBCs membrane by antioxidant effects so far reported.

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