Oxidative Stress and Erythrocyte Integrity in End-Stage Renal Failure Patients Hemodialysed Using a Vitamin E-Modified Membrane

Justin Westhuyzen,1 David Saltissi,2 and Veronica Stanbury 2
1Conjoint Renal Laboratory, Queensland Health Pathology Service, Royal Brisbane Hospital, and
2 Department of Renal Medicine, Royal Brisbane Hospital, Brisbane, Queensland, Australia

Abstract. Oxidative stress has been implicated in a range of disease states, including end-stage renal failure treated with hemodialysis. Hemodialysis with vitamin E-modified membranes reduces lipid peroxidation, but the effect on erythrocyte integrity has not been determined. This study compared antioxidant defense parameters and the resistance of erythrocytes to free radical-mediated hemolysis in patients dialysed with cellulose acetate membranes at baseline and with a vitamin E-modified membrane (Excebrane Clirans; Terumo Corporation) for 13 wk. Resistance of erythrocytes to free radical attack was assessed in vitro using the peroxyl hemolysis test. The time to 50% hemolysis (T50%) increased significantly during the first 6 wk of Excebrane use (p <0.05), but this parameter returned to baseline by 13 wk. Glutathione concentration and erythrocyte superoxide dismutase activity were unchanged during the study, but glutathione peroxidase activity increased from low levels at baseline and became significantly higher at 6 and 13 wk (p <0.001). Total erythrocyte polyunsaturated fatty acid content and C18:2 level increased (p <0.001) and saturated fatty acids (total, C16:0, C18:0, C22:0 and C24:0) decreased (p <0.03). Total monounsaturated fatty acid content was unchanged. The increased resistance of erythrocytes to hemolysis, the increased glutathione peroxidase activity, and the increased degree of unsaturation of fatty acids in the erythrocyte membrane are compatible with a reduction of oxidative stress during hemodialysis with vitamin E-modified membranes.

Introduction

End-stage renal failure (ESRF) treated with maintenance hemodialysis is associated with shortened red cell survival and increased hemolysis [1,2]. Since dialyser surface chemistry is important in blood-membrane interactions, more biocompatible membranes might lead to less dialysis-related hemolysis through reduced generation of damaging reactive oxidative species, such as oxygen derived free radicals (ODFR), and reduced oxidative stress.

Oxidative stress has been described as the imbalance between pro-oxidant forces and antioxidant activity in favor of the former [3,4]. Lipids are key targets. Polyunsaturated fatty acids (PUFA) undergo lipoperoxidation, as evidenced by increased levels of breakdown products like malondialdehyde (MDA) in erythrocytes and plasma [5-9]. Increased lipoperoxidation has implications not only for atherosclerosis, but also for stability and integrity of the red cell membrane through disruption of membrane architecture.

Recently, a dialyser membrane coated with the major lipophilic antioxidant, α-tocopherol (vitamin E), has been developed (Excebrane Clirans; Terumo Corp.). Studies suggest that Excebrane reduces the generation of ODFR both in vitro and in vivo [10-12], with reductions in plasma and erythrocyte lipid peroxidation. The membrane is coated with vitamin E (Excebrane Clirans; Terumo Corp.), which is known to reduce lipid peroxidation in vitro and in vivo [10-12]. The membrane is designed to reduce the generation of ODFR and improve the biocompatibility of the dialyser surface.
peroxidation [13]. While recombinant human erythropoietin (rHuEPO) therapy is known to increase red cell survival substantially [14], any contribution of a bio-compatible dialyser to increased red cell survival is undetermined.

Insights into the state of the red cells can be obtained indirectly by examining their susceptibility to free radical-induced hemolysis in vitro [15]. In the peroxyl hemolysis test, washed erythrocytes are incubated with a free radical generator, 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH), and the time to 50% hemolysis determined from a sigmoidal plot of hemolysis versus time. In the present study, a cohort of stable patients receiving recombinant human erythropoietin (rHuEPO) and dialysed with cellulose acetate dialysers was switched to vitamin-E coated Excebrane dialysers and followed for 13 wk. Antioxidant defenses, erythrocyte polyunsaturated fatty acid composition, and resistance to free radical-mediated hemolysis were determined before and after dialysis.

Methods

The protocol was approved by the Human Ethics Committee of the Royal Brisbane Hospital. Written, informed consent was obtained from participants.

Subjects. Twelve stable ESRF patients (7 men, 5 women, age 26 to 84 yr (mean 69.2 ±15.6 yr), were enrolled in this open, prospective 13-wk study. The patients were being dialyzed 3x/wk with cellulose acetate dialysers and receiving rHuEPO. None had evidence of active blood loss, blood dyscrasias, or bleeding disorder, or had received blood transfusion within two mo prior to the study. None had evidence of chronic infection or chronic inflammatory disease, as indicated by a C-reactive protein level >15 mg/L (3x the upper limit of the reference range), active malignant disease, uncontrolled diabetes mellitus (HbA1c level >8.0%), or uncontrolled thyroid disease. They were not receiving treatments with immunosuppressive drugs, antioxidants, or other confounding therapies.

All patients received erythropoietin (Eprex, Janssen-Cilag, Sydney, Australia). Eight of the 12 were supplemented with one tablet/day of Vitamin B Group Forte (Fawns & McAllan, Clayton, Victoria, Australia), which contains 5 mg vitamin B1 (thiamine), 2.5 mg vitamin B2 (riboflavin), 25 mg nicotinamide, 37.5 mg vitamin C (ascorbic acid), 0.5 mg vitamin B6 (pyridoxine), and 1 mg pantothenic acid/tablet. Seven subjects also received folic acid supplements (300 to 500 µg/day). Three subjects were supplemented with oral iron (270 mg ferrous sulphate/day), another 6 received iv iron polymaltose complex (50 mg/week) (Ferrum H, Sigma Pharmaceuticals).

Six apparently healthy adult subjects with normal renal function served as controls for the peroxyl hemolysis test.

Procedure. After baseline studies using cellulose acetate dialysers (Tuta-Nipro FB 210T or FB 150T), patients were switched to the vitamin E-modified, Excebrane membrane (Clirans CLE15NL, CLE-18NL, or CLEE18NL; Terumo Corp.) for 13 wk. Blood samples were drawn from the arterial line of the dialyser before and immediately after a routine dialysis session at 3 time points (baseline, 6 wk, and 13 wk).

Analytical methods. The resistance of erythrocytes to free radical attack was assessed in vitro using the peroxyl hemolysis test, as described by Boda [15], except that a 1% red cell suspension (rather than 0.5%) was used. The red cell suspension was prepared and mixed with an equal volume of the free radical generator, 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH, 74 mM). Two tubes per sample, representing a test sample and a total hemolysis sample, were incubated at 37°C with gentle shaking in air for 150 min. Aliquots were removed every 30 min and added to equal volumes of phosphate buffered saline (PBS) (test sample) or 0.1% Triton X-100 (total hemolysis). Mixtures were centrifuged immediately (1500 x g, 5 min) and the absorption of supernatant hemoglobin determined spectrophotometrically at 540 nm (DU650 spectrophotometer, Beckman Instruments, Fullerton, CA, USA). The % hemolysis at each time point was calculated as the ratio of the test sample to the total hemolysis sample. The time to 50% hemolysis (T50%) was derived from a sigmoidal plot.
of time vs % hemolysis. The antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx), were measured spectrophotometrically on a Cobas-Bio analyser (Roche, Basel, Switzerland) using RANSOD and RANSEL kits (Randox Laboratories, Crumlin, UK). Reduced glutathione was measured spectrophotometrically by an adaptation of the method of Beutler et al [16], as described by Konukoglu et al [17].

For erythrocyte fatty acid analysis, heparinised blood was centrifuged (1125 x g; 12 min; 4 °C) and plasma and buffy coat were removed. Erythrocytes were washed 3x with isotonic buffered saline (PBS). An aliquot of packed red cells (250 µl) was lysed by freezing at -85°C for 25 min followed by 10 min at 37°C. Total lipids were extracted from the hemolysed cells with 4.5 mL of isopropanol/chloroform (11:7, v/v) by sequentially adding 2.75 ml of isopropanol with vortexing, followed by addition of 1.75 ml of chloroform [18]. After centrifugation, the supernate was evaporated to dryness under nitrogen and fatty acids were esterified by the direct method of Lepage and Roy [19]. Fatty acid methyl esters were separated using a Varian-3500 capillary gas chromatograph (Varian Corp., Walnut Creek, CA, USA) fitted with a moderately polar BP225 capillary column (25 m x0.53 mm internal diameter; SGE, Melbourne, Australia). On-column injection and flame ionisation detection were used with hydrogen as the carrier gas. Column temperature was increased at 50°C/min from an initial 80°C to 180°C, then at 1°C/min to 205°C; after 2 min, the temperature was increased at 5°C/min to 215°C; the final hold time was 20 min. The injector was programmed to increase from 90°C to 230°C at 200°C/min; the flame ionisation detector was used at 240°C. Peaks were identified by comparison with reference standards (Alltech Associates, Deerfield, IL, USA). Chromatograms were plotted and the peak areas were calculated using an automatic integrator (LDC/Milton Roy, Riviera Beach, FL, USA). Fatty acid data were expressed as % of total fatty acids.

Iron was estimated spectrophotometrically using automated analysers (Hitachi 747 and Cobas-Fara (Hoffman LaRoche, Basel, Switzerland)) and a reagent kit (Boehringer-Mannheim, Germany). Immunonephelometric methods were used to assay serum transferrin, iron binding capacity (TIBC), and ferritin (Nephelometer Analyzer II, Behring Corporation, Marburg, Germany). Hematological parameters were measured using a Sysmex-9000 cell counter (Kobe, Japan) with standard calibration.

**Data analysis.** Data are described as means ±SD

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Table 1. Erythropoietin use, iron status, and hematological variables during the study; means ± SD or medians (and interquartile ranges)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>At baseline</th>
<th>At 6 wk</th>
<th>At 13 wk</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>rHuEPO use</td>
<td>IU/wk</td>
<td>6,000 (4,000-12,000)</td>
<td>7,000 (5,000-15,000)</td>
<td>7,000 (4,000-15,000)</td>
<td>ns</td>
</tr>
<tr>
<td>Serum iron</td>
<td>µmol/L</td>
<td>12.2 ±4.3 (22.0-29.5)</td>
<td>10.6 ±4.7 (16.0-29.0)</td>
<td>10.8 ±4.5 (16.0-32.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Serum transferrin saturation</td>
<td>%</td>
<td>25.5 (22.0-29.5)</td>
<td>19.5 (16.0-29.0)</td>
<td>22.5 (16.0-32.0)</td>
<td>ns</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>µg/L</td>
<td>544 (294-674)</td>
<td>572 (311-600)</td>
<td>434 (302-574)</td>
<td>ns</td>
</tr>
<tr>
<td>Blood hemoglobin</td>
<td>g/dL</td>
<td>10.9 ±1.0 (10.9-12.0)</td>
<td>11.1 ±1.5 (11.0-12.0)</td>
<td>10.9 ±1.6 (10.8-12.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Blood red cell count</td>
<td>x10&lt;sup&gt;12&lt;/sup&gt;/L</td>
<td>3.37 ±0.32 (3.30-3.40)</td>
<td>3.52 ±0.49 (3.35-3.60)</td>
<td>3.55 ±0.51 (3.30-3.70)</td>
<td>ns</td>
</tr>
<tr>
<td>Blood hematocrit fraction</td>
<td></td>
<td>0.337 ±0.027</td>
<td>0.347 ±0.049</td>
<td>0.343 ±0.050</td>
<td>ns</td>
</tr>
</tbody>
</table>

<sup>a</sup> RM-ANOVA or RM-ANOVA on ranks; ns, not significant (p >0.05)
for normally distributed data and as medians with interquartile ranges for skewed data. Pre- and post-dialysis measurements were compared by the paired t-test. Sequential data were analysed by repeated measures analysis of variance (RM-ANOVA) or repeated measures ANOVA on ranks for skewed data; pairwise multiple comparisons were performed by the Tukey test. A two-tailed probability p < 0.05 was considered significant.

Results

Eleven of the 12 patients received Excebrane dialysis according to prescription for the duration of the study without complications. One patient (a 69 yr old man who received 20,000 IU rHuEPO/wk) displayed excessive clotting using an EE18 Excebrane dialyser with both unfractionated heparin and low-molecular weight heparin anticoagulation. He was consequently switched back to a standard cellulose acetate membrane.

Iron status and hematological variables are summarised in Table 1. The rHuEPO doses were stable throughout the study period. There were no significant changes in iron indices (serum iron, transferrin saturation, ferritin), nor in blood hemoglobin, erythrocyte count, or hematocrit during the study period.

Antioxidant defense parameters and susceptibility of erythrocytes to free radical induced hemolysis in vitro are shown in Table 2. There were no significant differences pre- and post-dialysis for any of the parameters (p >0.05). Glutathione level and erythrocyte SOD activity were unchanged during the study. GPx increased from low levels at baseline and was significantly higher at 6 and 13 wk (p <0.001). The time to 50% hemolysis in vitro increased significantly during the first 6 wk (p <0.05) and was significantly longer than that in the group of normal controls (84.8 ±11.3 versus 75.8 ±7.2 min, respectively; p = 0.015). The T50% time (predialysis) was correlated with SOD activity at baseline (r = 0.609; p = 0.036) and at 6 wk (r = 0.620; p = 0.042), but not with any of the other antioxidant parameters. By 13 wk, T50% returned to baseline (Table 2). There was negative correlation that approached significance between T50% (predialysis) and rHuEPO dose with cellulose acetate membrane (baseline r = -0.55; p = 0.064), but not with Excebrane (r = -0.35, p >0.3), suggesting that red blood cells may be less susceptible to hemolysis with the latter.

The erythrocyte fatty acid composition is

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Table 2. Effect of dialysis with Excebrane for 13 wk on erythrocyte antioxidant status and susceptibility to free radical induced hemolysis in vitro; means ±SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Pre/post</th>
<th>At baseline</th>
<th>At 6 wk</th>
<th>At 13 wk</th>
<th>p a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glutathione</td>
<td>mmol/L</td>
<td>Pre</td>
<td>0.99 ±0.18</td>
<td>1.06 ±0.21</td>
<td>1.06 ±0.20</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>0.99 ±0.17</td>
<td>1.08 ±0.16</td>
<td>1.04 ±0.20</td>
<td>ns</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>U/g Hb</td>
<td>Pre</td>
<td>781 ±124</td>
<td>745 ±134</td>
<td>801 ±202</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>725 ±102</td>
<td>746 ±130</td>
<td>771 ±151</td>
<td>ns</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>U/g Hb</td>
<td>Pre</td>
<td>46.5 ±14.9</td>
<td>55.6 ±14.3*</td>
<td>56.1 ±15.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>46.8 ±14.8</td>
<td>55.7 ±14.8*</td>
<td>56.7 ±17.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemolysis T50%</td>
<td>min</td>
<td>Pre</td>
<td>77.7 ±3.3</td>
<td>84.8 ±11.3*</td>
<td>72.9 ±3.1b¶</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post</td>
<td>78.3 ±6.9</td>
<td>81.0 ±8.3</td>
<td>nd</td>
<td>ns</td>
</tr>
</tbody>
</table>

a One-way repeated measures analysis of variance (RM-ANOVA).

b Tested at 17 wk.

* Significantly different from baseline, p <0.05.

¶ Significantly different from 6 wk, p <0.05.

ns: not significant; nd: not done.
summarised in Table 3. Total saturated fatty acids (ΣSFA) (p <0.001) and the fatty acids 16:0 (palmitic acid), 18:0 (stearic acid), 22:0 (behenic acid), and 24:0 (lignoceric acid) were all significantly lower in patients while on Excebrane dialysis. Although total monounsaturated fatty acid (ΣMUFA) content was unchanged, C18:1 (oleic acid) was increased and C24:1 (nervonic acid) was decreased (p <0.001). Total polyunsaturated fatty acids (ΣPUFA) and C18:2 (linoleic acid) were increased significantly (p <0.001). Changes in arachidonic acid (C20:4) and eicosapentanoic acid (C20:5; EPA) did not reach statistical significance. The time to 50% hemolysis (T₅₀%) did not correlate with ΣSFA, ΣMUFA, or ΣPUFA (predialysis samples). However, at baseline (predialysis), T₅₀% was correlated negatively with erythrocyte C20:4 content (r = -0.654; p = 0.029).

Table 3. Effect of dialysis with Excebrane for 13 wk on erythrocyte fatty acid composition (area %; mean ±SD).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Controls n = 6</th>
<th>At baseline</th>
<th>At 6 wk</th>
<th>At 13 wk</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>24.39 ±1.53</td>
<td>22.78 ±0.78‡</td>
<td>23.39 ±0.96</td>
<td>22.57 ±1.61‡</td>
<td>0.024</td>
</tr>
<tr>
<td>18:0</td>
<td>15.15 ±0.88</td>
<td>16.94 ±0.65‡</td>
<td>15.61 ±0.66 *</td>
<td>15.52 ±1.25 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:1</td>
<td>15.93 ±0.83</td>
<td>15.54 ±0.92</td>
<td>16.10 ±1.02</td>
<td>17.02 ±1.48 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:2</td>
<td>9.18 ±1.48</td>
<td>8.56 ±1.00</td>
<td>9.22 ±0.96</td>
<td>10.49 ±2.88 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20:4</td>
<td>17.43 ±0.53</td>
<td>16.74 ±0.72</td>
<td>17.15 ±1.17</td>
<td>17.20 ±1.29</td>
<td>ns</td>
</tr>
<tr>
<td>20:5</td>
<td>1.12 ±0.35</td>
<td>0.76 ±0.26‡</td>
<td>0.65 ±0.30‡</td>
<td>0.68 ±0.24‡</td>
<td>ns</td>
</tr>
<tr>
<td>22:0</td>
<td>0.83 ±0.11</td>
<td>1.03 ±0.17</td>
<td>0.87 ±0.21 *</td>
<td>0.69 ±0.15 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:4</td>
<td>2.71 ±1.18</td>
<td>3.33 ±0.63</td>
<td>3.37 ±0.31</td>
<td>3.28 ±0.49</td>
<td>ns</td>
</tr>
<tr>
<td>22:6</td>
<td>8.29 ±0.94</td>
<td>7.26 ±0.90</td>
<td>7.18 ±0.77</td>
<td>8.02 ±2.26</td>
<td>ns</td>
</tr>
<tr>
<td>24:0</td>
<td>2.33 ±0.34</td>
<td>3.08 ±0.55‡</td>
<td>2.71 ±0.61 *</td>
<td>1.90 ±0.39 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24:1</td>
<td>2.38 ±0.41</td>
<td>3.93 ±0.81‡</td>
<td>3.59 ±0.79 *‡</td>
<td>2.43 ±0.73*‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΣSFA</td>
<td>42.70 ±1.35</td>
<td>43.83 ±0.77</td>
<td>42.57 ±1.49 *</td>
<td>40.67 ±1.93 *‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>18.32 ±1.04</td>
<td>19.47 ±1.19</td>
<td>19.69 ±1.24</td>
<td>19.45 ±1.59</td>
<td>ns</td>
</tr>
<tr>
<td>ΣPUFA</td>
<td>38.99 ±1.74</td>
<td>36.70 ±1.47</td>
<td>37.74 ±1.65 *</td>
<td>39.88 ±3.03 *‡</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Significance shown for repeated measures analysis of variance comparisons for dialysis patients; the Tukey test was used for pairwise multiple comparisons.
‡ Significantly different from controls, p <0.05.
* Significantly different from baseline, p <0.05.
¶ Significantly different from 6 wk, p <0.05.
§ Significantly different from 6 wk, p <0.05.

Discussion

Erythrocyte integrity and resistance to hemolysis during dialysis are determined by factors such as antioxidant reserve, including levels of antioxidant enzymes, and cell structure, especially cell wall composition. We have examined aspects of these relationships following dialysis with the vitamin E-coated membrane, and have described compositional changes in the fundamental building blocks of the erythrocyte membrane, the fatty acids, with significant increases in the PUFA content and
decreases in the SFA fraction. These changes have implications for membrane fluidity, susceptibility to free radical attack, and, by inference, erythrocyte integrity.

The significant increase in resistance to free radical attack that occurred in the initial weeks after switching to Excebrane is suggestive of a reduction of oxidative stress. This change in resistance occurred despite the increase in unsaturation of red cell membrane fatty acids (which theoretically increases the targets for free radical attack). Moreover, the effect was unaccompanied by changes in intracellular antioxidants, apart from GPx. The protection afforded against free radical induced hemolysis may therefore be due (at least in part) to the vitamin E residues on the modified membrane, resulting in sparing of the endogenous vitamin E pool and augmentation of antioxidant reserve [13].

It has been suggested [20] that the halving in number of dysmorphic erythrocytes (echinocytes) following Excebrane use for 1 mo could be due to improvement in red cell membrane characteristics linked to the effect of the vitamin E. The Excebrane membrane contains 140 mg/m² of vitamin E [21], which is substantial in comparison with plasma vitamin E levels in dialysis patients (10 to 20 mg/L; 25 to 46 µM; MW 430). Clearly, the potential for free radical scavenging and antioxidant activity is significant, even given that the α-tocopherol residues are immobilised on the Excebrane membrane.

Clinical implications of reduced susceptibility to hemolysis might be increased red cell survival and lower rHuEPO use. Significantly increased red cell survival following dialysis with Excebrane dialysers for 10 mo has been reported in patients receiving both rHuEPO and glutathione (1.2 g, iv, after each dialysis session)[22]. Moreover, EPO use was reduced to 51% of the initial dose without negative effects on hemoglobin and erythrocyte count. However, in our study, no substantial changes in rHuEPO requirement were noted.

The erythrocyte is enucleated when it enters the circulation, and hence contains its full complement of macromolecules, enzymes, and lipids. While no induction of antioxidant enzymes is possible in mature erythrocytes, induction of specific proteins could occur during erythropoiesis in the bone marrow [23]. Interestingly, although reduced glutathione and erythrocyte SOD activity were unchanged during the study, GPx increased from low levels at baseline and was significantly higher at 6 and 13 wk (p <0.001). Since enzyme levels were low at baseline, this change may represent a “normalisation” of activity levels afforded by a reduction in systemic oxidative stress. Mydlik et al [21] also reported an increase in erythrocyte GPx activity after dialysis with vitamin E-modified membranes (means for 8 subjects of 54.3 ±8.1 U/g Hb on conventional Clirans dialyser and 62.7 ±7.2 U/g Hb after 10 dialyses with vitamin-E modified Clirans dialyser). Although stimulation of erythropoiesis leads to a younger population of erythrocytes that have higher enzyme activities [24], our patients were stable on EPO and no changes in hematological indices were noted during the study.

GPx protects PUFA by removal of lipoperoxides formed by free radical attack of susceptible unsaturated double bonds, such as are present in high concentrations in the cell membrane. The increases in both PUFA and the protective enzyme GPx in the red cells in our study may therefore be linked. The fatty acid composition of the red cells is initially determined by the pool of available fatty acids in the marrow during erythropoiesis, but this can be modified in the circulation by exchange processes with fatty acids carried by serum lipids [25].

Although the changes in erythrocyte fatty acid composition, and in particular the increase in total PUFA, may reflect dietary influences, the patients’ diets were stable and a change of the order observed is unlikely. It is more likely that the shift from saturated to unsaturated moieties is due to increased antioxidant protection from the vitamin E-modified dialyser, as noted by Galli et al [13]. Contribution of the dialyser to antioxidant activity has also been demonstrated by reduced MDA level in erythrocytes after dialysis with vitamin E-coated dialysers [9]. Recently, Galli et al [26] showed that vitamin E supplementation of dialysis patients with borderline vitamin E-deficiency was associated with significant increases in PUFA and decreases in SFA of plasma lipids, while the relative concentration of MUFA was unchanged. These findings closely parallel the findings in our study regarding erythrocyte fatty acid
composition after Excebrane dialysis, and together point to a significant antioxidant effect of additional vitamin E (bound or unbound) in HD patients [26].

Few studies have experimentally addressed the susceptibility of erythrocytes to hemolysis in hemodialysis patients. Zachhée et al [24] examined the oxidative sensitivity of red blood cells using a modified ascorbate-cyanide test. The test measured the accumulation of methemoglobin within the red cells after 90 min incubation, but did not continue to the end-point of hemolysis. Met-Hb levels in their patients were much higher than in controls, but were not influenced by erythropoietin administration. Ono [27] described increased osmotic fragility of erythrocytes from dialysis patients versus normal subjects. Interestingly, vitamin E supplementation (600 mg/day for 30 days) significantly reduced the susceptibility of red cells to osmotic hemolysis in their dialysis patients.

In the present study, no significant changes were noted during the dialysis procedure (pre- versus post-dialysis) for any of the antioxidant defense parameters studied, nor in the susceptibility of erythrocytes to free radical-induced hemolysis in vitro (p >0.05). Previously, we reported a diminution of total antioxidant status (TAS) during dialysis, which was attributed to dialytic loss of low molecular weight antioxidants such as ascorbate, bilirubin, and urea [28]. In contrast, plasma vitamin A and E concentrations increased slightly, reflecting the effect of hemoconcentration during dialysis [28]. Other workers have reported little or no change in plasma vitamin A and E levels after a single dialysis session [21,29], but Usberti et al [22] reported 17% increase in plasma vitamin E levels using non-vitamin E-modified membranes, and 14% increase using the CL-E (vitamin E-modified) dialyser. The reason for the increase in vitamin E levels (when observed) is unclear, but probably involves correction of any nutritional deficiency and overall improvement in antioxidant capacity of the subjects due to the antioxidant activity reserve afforded by the vitamin E-modified membrane, with sparing of the endogenous vitamin E pool [13].

In summary, we find dialysis with vitamin E-modified membranes to be associated with a significant but temporary increase in resistance of isolated, washed red cells to free radical-induced hemolysis in vitro that is accompanied by increases in GPx and in the degree of unsaturation in fatty acids of erythrocyte membranes. Changes in other erythrocyte antioxidants were not observed. These results are compatible with a reduction in oxidative stress during dialysis with vitamin E-modified membranes.

Acknowledgments

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