Hemodialysis Impairs Endothelial Function via Oxidative Stress
Effects of Vitamin E–Coated Dialyzer

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Background—Patients who undergo hemodialysis experience accelerated atherosclerosis and premature death. Recent evidence suggests that endothelial dysfunction proceeds to and exacerbates atherosclerosis. It remains unknown whether hemodialysis per se causes endothelial dysfunction.

Methods and Results—We evaluated endothelial function estimated by flow-mediated vasodilation during reactive hyperemia using high-resolution ultrasound Doppler echocardiography before and after a single session in patients on maintenance hemodialysis. Several studies have shown that the imbalance between pro-oxidant and antioxidant activities in hemodialyzed patients results in high oxidative stress, which causes lipid peroxidation and endothelial injury. Accordingly, we investigated the effects of antioxidative modification during hemodialysis on endothelial function using a vitamin E–coated cellulose membrane dialyzer. Nonspecific endothelium-independent vasodilation was measured after administration of a sublingual glyceryl trinitrate spray (0.3 mg). A single session of hemodialysis by noncoated dialyzer impaired flow-mediated vasodilation \((P<0.05)\) associated with increased plasma levels of oxidized LDL \((P<0.05)\), an index of oxidative stress. Hemodialysis by vitamin E–coated membrane prevented dialysis-induced endothelial dysfunction and increases in oxidized LDL. Plasma levels of oxidized LDL were inversely correlated with the magnitudes of flow-mediated vasodilation \((r=−0.53, P<0.001)\). Hemodialysis by noncoated or vitamin E–coated membrane did not affect glyceryl trinitrate–induced endothelium-independent vasodilation.

Conclusions—Our findings indicate that hemodialysis per se impairs endothelial function, possibly by increasing oxidative stress. (Circulation. 2000;101:1002-1006.)

Key Words: antioxidants ■ atherosclerosis ■ vasodilation ■ kidney ■ lipoproteins

Patients with end-stage renal diseases who undergo hemodialysis experience accelerated atherosclerosis, and the high prevalence of atherosclerotic cardiovascular death accounts for a great majority of mortality in this population.1–4 Morphological and mechanical alterations of conduit arteries have been characterized by atherosclerosis in hemodialyzed patients.5–9 Endothelium-derived NO plays a crucial role not only in control of vascular tone of conduit arteries but also in antiatherosclerosis by several mechanisms, such as inhibition of platelet aggregation/adhesion or of smooth muscle cell proliferation.10–12 Recent reports demonstrated that NO-dependent flow-mediated vasodilation is blunted in patients undergoing hemodialysis,13,14 suggesting that the impaired bioactivity of endothelium-derived NO in the conduit artery may contribute to the progression of atherosclerosis in hemodialyzed patients.

Oxidative stress plays a pivotal role in the pathogenesis of vascular injury and in the progression of atherosclerosis by several mechanisms, some of which are associated with the inhibition of NO synthase activity and the inactivation of NO by reactive oxygen species.15 Previous studies have shown the imbalance in pro-oxidant and antioxidant activities in hemodialyzed patients,16 resulting in high oxidative stress demonstrated as enhanced lipid peroxidation.17,18 Hemodialysis per se has been suggested to induce oxidative stress, with reactive oxygen species being generated on the surface of dialysis membranes by activation of polymorphonuclear leukocytes.19–23 Indeed, it has been well documented that even a single session of hemodialysis significantly increases lipid peroxides and decreases antioxidants.24–27 Thus, it is plausible that hemodialysis induces oxidative stress and impairs the bioactivity of NO, resulting in accelerated atherosclerosis.

To attenuate the activation of polymorphonuclear leukocytes on the surface of dialysis membranes, a vitamin E–coated multilayer hemodialysis filter was recently produced.28,29 With this dialyzer, it has been shown that the
consumption of blood antioxidants, oxidative demolition of lipids, and activation of leukocytes were significantly attenuated.28,29

Accordingly, we hypothesized that hemodialysis per se may impair endothelial function and that hemodialysis using a vitamin E–coated dialyzer may restore endothelial dysfunction. To test this hypothesis, we measured flow-mediated vasodilation and plasma levels of oxidized LDL (oxLDL), an index of lipid peroxidation, before and after a single session of hemodialysis by a crossover design using vitamin E–coated or noncoated cellulose membrane dialyzer.

Methods

Subjects

Twelve hemodialyzed patients (6 men, 6 women) were enrolled in our study. All patients were receiving hemodialysis 3 times a week by cellulosic membrane dialyzer, and each session lasted for 4 to 5 hours. They had been undergoing dialysis for a mean of 6.5 ± 2.9 years. Causes of end-stage renal disease were glomerulonephritis (n = 10), interstitial nephritis (n = 1), and Fanconi syndrome (n = 1). Patient mean age was 40.5 ± 3.6 years (range, 20 to 64 years). All female patients were postmenopausal. No patients had symptomatic coronary artery diseases or a family history of premature cardiovascular diseases. All subjects were nonsmokers, nonobese (body mass index, 21.1 ± 0.4 kg/m²), nondiabetic (fasting plasma glucose, 93 ± 4 mg/dL), and normallipidemic (total cholesterol, 151 ± 7 mg/dL; LDL cholesterol, 82 ± 6 mg/dL; HDL cholesterol, 47 ± 3 mg/dL; triglycerides, 106 ± 10 mg/dL), with normal plasma levels of vitamin E (0.91 ± 0.05 μmol/mL). All patients were treated with a small dose of erythropoietin (3000 U/wk). Six patients were hypertensive and were taking a calcium antagonist (n = 4), a calcium antagonist plus ACE inhibitor (n = 1), a calcium antagonist plus ACE inhibitor plus α-blocker (n = 1), and an antiplatelet agent (n = 1). All medications were discontinued 12 hours before the study.

General Procedure

The protocol was explained, and written informed consent was obtained from each subject. The study was approved by the Ethical Committee for Human Study in our institution. The study was done with subjects in the supine position and in an air-conditioned room at 22°C to 23°C. We used a noncoated cellulose dialyzer (CLS-15N) or a cellulose dialyzer coated with the antioxidant vitamin E (CLE-15N) of the same membrane size. For the first experiment, all subjects underwent hemodialysis by either a cellulose or a vitamin E–coated cellulose membrane dialyzer. Endothelial function and blood chemistry measurements were done immediately before and after hemodialysis. Then they underwent hemodialysis twice by cellulose membrane. For the second experiment, the protocol of the first experiment was repeated in all patients by a crossover design. Blood samples were obtained from the contralateral shunt arm vein.

Measurements of Endothelial Function

Flow-mediated vasodilation of the brachial artery was measured by a previously described noninvasive technique to assess endothelial function.30 Briefly, after a 10-minute equilibration period, with the use of a 10-MHz linear-array transducer and the SSA-380A system (Toshiba), the brachial artery was longitudinally imaged using a 10-MHz linear-array transducer and the SSA-380A system, twiced at baseline and then every 15 minutes continuously after the release of 4.5-minute upper arm cuff occlusion. Arterial blood flow was determined as arterial cross-sectional area times mean Doppler velocity. The magnitude of reactive hyperemia was calculated as the maximum flow divided by the baseline flow. As an internal control, we measured changes in the brachial diameter, ie, endothelium-independent vasodilation, induced by sublingual glyceryl trinitrate (GTN; 300 μg; Myocol Spray, Toa Eiyo Co) given 15 minutes after the measurement of flow-mediated vasodilation. Five minutes after GTN administration, the scan was done to assess endothelium-independent vasodilation.

Oxidized LDL

Blood samples were collected in a tube containing EDTA, and plasma was stored in a refrigerator at 4°C. Plasma oxLDL was measured by a sandwich ELISA method as previously described.17,31,32 Briefly, LDL fractions were obtained from samples by sequential ultracentrifugation. The diluted LDL fractions (5 μg/well) were added to microtiter wells precoated with 0.5 μg of an anti-oxLDL monoclonal antibody (FOH1a/DLH3). After extensive washing, the remaining oxLDL was detected by use of a sheep anti-human apolipoprotein B antibody and an alkaline phosphatase–conjugated anti-sheep IgG antibody. In each ELISA plate, various concentrations of standard oxLDL, which was prepared by inactivating LDL with 5 μmol/L CuSO₄ at 37°C for 3 hours, were run simultaneously to determine a standard curve.

Other Chemical Analyses

Plasma vitamin E was determined by high-performance liquid chromatography. Serum total cholesterol, HDL cholesterol, triglycerides, creatinine, blood urea nitrogen, and glucose were determined with commercial kits (Boehringer Diagnostica, Wako Chemicals Co, Daichi Chemicals Co, and Kanto Chemical Co). LDL cholesterol was calculated by Friedewald’s formula.

Statistical Analysis

Results were expressed as mean ± SEM, and a value of P < 0.05 was considered to be statistically significant. Predialysis and postdialysis results with noncoated or vitamin E–coated dialyzer were analyzed by 2-way ANOVA for repeated measures. Linear regression analysis was performed between oxLDL and flow-mediated dilatation.

Results

A single session of dialysis with noncoated or vitamin E–coated dialyzer similarly decreased body weight, blood urea nitrogen, and creatinine and increased hematocrit (Table 1). Plasma levels of vitamin E were not different at baseline and were not altered by noncoated or vitamin E–coated dialyzer (Table 1). There were similar tendencies toward decreases in blood pressure and increases in heart rate after a session of hemodialysis by noncoated or vitamin E–coated dialyzer (Table 2).

Plasma OxLDL

Plasma levels of oxLDL before a session of hemodialysis were similar between noncoated and vitamin E–coated dialyzers (P = NS, Figure 1). After a session of hemodialysis by noncoated cellulose dialyzer, oxLDL increased by 35% from baseline (P < 0.05; Figure 1), whereas it was not altered by vitamin E–coated dialyzer (P = NS by 1-way ANOVA; P < 0.05 versus noncoated dialyzer by 2-way ANOVA; Figure 1).
Vascular Function

Before and after hemodialysis, there were no significant differences in the baseline vessel diameter, peak hyperemic response, or GTN-induced vasodilation between noncoated and vitamin E–coated dialyzers (Table 2). After a session of hemodialysis by noncoated cellulose dialyzer, flow-mediated vasodilation was significantly blunted (P<0.05; Figure 2). By contrast, the vitamin E–coated dialyzer did not alter flow-mediated dilation (P=NS by 1-way ANOVA; P<0.005 versus noncoated dialyzer by 2-way ANOVA; Figure 2). Furthermore, plasma levels of oxLDL were inversely correlated with the magnitudes of flow-mediated vasodilation (r=-0.53, P<0.001; Figure 3). There were no significant differences in the basal diameters of the brachial artery, peak hyperemic responses, flow-mediated vasodilation, or GTN-induced vasodilation between hypertensives and normotensives or between men and women, and these parameters were not affected by the use of medications (data not shown). There was no correlation between GTN-induced vasodilation and plasma oxLDL.

Discussion

The salient findings of this study are that (1) endothelium-dependent vasodilation was blunted after a single session of hemodialysis by cellulose membrane dialyzer, associated with the increase in plasma oxLDL, an index of oxidative stress, and (2) with the use of a vitamin E–coated cellulose membrane dialyzer, hemodialysis-induced endothelial dys-
have consistently advocated that NO production during he-
modialysis is increased rather than decreased 37–39 and endog-
enous NO synthase inhibitors are eliminated by hemodialy-
sis.34 Hemodialysis may inactivate NO by generating reactive
oxygen species on the surface of dialysis membranes by
activation of polymorphonuclear leukocytes.19 –23 To address
this issue, we measured plasma levels of oxLDL, which were
significantly elevated after a single session of hemodialysis.
OxLDL is not only an index of lipid peroxidation but also
causes endothelial dysfunction by itself through the impair-
ment of the signal transduction between endothelial cell
surface receptors and NO production,40 inhibition of NO
synthase activity, 40 and inactivation of NO released from
endothelial cells. 40 We have previously reported that rapid
removal of oxLDL by a single session of apheresis improves
endothelium-dependent vasodilation in hypercholesterolemic
patients.32 Thus, it may be possible that oxidation of LDL
during hemodialysis may directly impair endothelial function
and decrease the biological activity of NO. Our results might be
contrary to the findings by Hand et al,41 who demonstrated that
hemodialysis significantly improved acetylcholine-stimulated
vasodilation in the hand vein. Differences in vascular beds,42 ie,
conduit artery versus vein, or differences in vasodilatory stimu-
li,43 ie, physiological shear stress versus pharmacological ace-
tylcholine, may account for the discrepancy.

Effects of Vitamin E–Coated Dialyzer
To further examine the role of oxidative stress, we studied the
effects of a vitamin E–coated hemodialysis filter on endothe-
lial function. With this dialyzer, the impairment of endothe-
lium-dependent vasodilation induced by hemodialysis was
completely abolished. The possible mechanism(s) to account
for the difference in noncoated and vitamin E–coated dialyz-
ers can be summarized as follows: (1) systemic hemodynamic
alteration, (2) removal of uremic substances, and (3) effects
on vascular reactivity. The first possibility is unlikely, be-
cause hemodynamic parameters after hemodialysis, ie, blood
pressure, heart rate, basal forearm blood flow, and brachial
artery diameter, were similar between noncoated and vitamin
E–coated dialyzers. The second possibility is also less likely,
because body weight was decreased and blood urea nitrogen
and creatinine were effectively removed in a similar manner.
The third possibility may account for the difference in the
results. In the present study, the increases in blood flow
during reactive hyperemia and GTN-induced vasodilation
after a session of hemodialysis were similar between non-
coated and vitamin E–coated dialyzers, indicating that the
differences in flow-mediated vasodilation between noncoated
and vitamin E–coated dialyzers could be attributed to the
distinct effects on endothelial function. As expected, this
endothelial protection by the vitamin E–coated dialyzer may
be due to elimination of oxidative stress, because increases in
plasma levels of oxLDL by hemodialysis were prevented by
the vitamin E–coated dialyzer, and endothelial function was
inversely correlated with plasma oxLDL. Plasma levels of vitamin E were similar before and after hemodialysis and
between the noncoated and vitamin E–coated dialyzers. These
findings support the notion that the vitamin E–coated dialyzer
may decrease oxidative stress through the mechanism on the
surface of the dialysis membrane but not by systemic antioxidant effects of vitamin E eluted from the dialyzer.

In summary, hemodialysis per se impairs endothelial function, possibly by increasing oxidative stress. The long-term antiatherogenic effects of the vitamin E–coated dialyzer in patients receiving maintenance hemodialysis remain to be elucidated.

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