Low-Density Lipoprotein (LDL)-Induced Monocyte-Endothelial Cell Adhesion, Soluble Cell Adhesion Molecules, and Autoantibodies to Oxidized-LDL in Chronic Renal Failure Patients on Dialysis Therapy

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Premature atherosclerosis is a major cause of morbidity and mortality in chronic renal failure patients undergoing dialysis. In this study, we compared autoantibodies to oxidized low-density lipoprotein (OX-LDL), soluble cell adhesion molecules (CAMs), and the effect of both LDL and OX-LDL on monocyte endothelial cell adhesion in chronic renal failure patients on hemodialysis (HD, n = 16) and peritoneal dialysis (PD, n = 17) compared with matched healthy control subjects (C, n = 17). In addition, we studied the effect of supplementation with RRR-alpha-tocopherol (AT) 800 IU/d for 12 weeks on the above measures. LDL and OX-LDL induced adhesion of U937 cells to cultured endothelium, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intracellular adhesion molecule-1 (sICAM-1), and soluble E-selectin (sE-selectin); autoantibodies to OX-LDL and markers of lipid peroxidation were determined before and after AT supplementation. Native LDL from PD patients induced greater monocyte-endothelial cell adhesion than LDL from C subjects (43.8% ± 17.0% *v* 25.3% ± 17.7%, respectively, *P* = .0028). OX-LDL from chronic renal failure patients on both PD and HD stimulated greater adhesion than OX-LDL from the C subjects (68.0% ± 18.5% and 57.6% ± 15.1% *v* 40.9% ± 17.3%, respectively, *P* < .01); OX-LDL from PD patients induced greater adhesion than that from HD patients (*P* < .01). Plasma methylglyoxal levels were significantly increased in both HD and PD groups, with higher levels in the HD group. Chronic renal failure patients on HD and PD also had higher levels of plasma sVCAM-1 and sE-selectin than C subjects (*P* < .01), indicating endothelial activation. Titers of autoantibodies to OX-LDL were not elevated in renal failure patients. Supplementation with AT 800 IU/d for 12 weeks, while resulting in significant enrichment with AT in LDL, did not have a significant effect on any of the parameters studied. This study makes the novel observation that the LDL of chronic renal failure patients on HD and PD appears to be potentially more atherogenic, since it induces greater monocyte-endothelial cell adhesion.

PREMATURE ATHEROSCLEROSIS is a major cause of morbidity and mortality in chronic renal failure patients undergoing dialysis.[1,2] Cardiovascular disease has been reported to account for more than half of all deaths in patients on hemodialysis (HD).[2] Even after stratification by age, gender, race, and the presence of diabetes, cardiovascular disease mortality in HD and peritoneal dialysis (PD) patients is 5 to 20 times higher than in the general population.[3,4]

Increased oxidative modification of low-density lipoprotein (LDL) has been implicated in the etiology of accelerated atherosclerosis in chronic renal failure.[5-8] Maggi et al[5,6] have reported elevated autoantibodies to oxidized LDL (OX-LDL), and two groups have found increased LDL susceptibility to oxidation.[5,7] Also, increased LDL degradation by macrophages[8] has been observed in patients on dialysis, although these findings have not been consistently confirmed by other investigators.[9-12] Oxidative stress,[13,14] depleted antioxidants such as alpha-tocopherol (AT),[15] dyslipidemia,[16-18] abnormal LDL composition,[16-19] and LDL glycation[20-24] may contribute to the increased oxidative modification and atherogenicity of LDL in chronic renal failure patients. However, based on current data, it is unclear whether there are significant differences in the degree of oxidative modification of LDL in chronic renal failure patients on HD and PD compared with a healthy population.

OX-LDL is cytotoxic[25] and has deleterious effects on the function of the vascular endothelium, resulting in inflammation and thrombosis, the hallmark of atherosclerosis.[26-28] In vitro studies have demonstrated that OX-LDL can increase the adhesion of monocytes to endothelial cells,[29] the purported initial step in atherogenesis. Native LDL and OX-LDL have been shown to induce expression of the adhesion molecules E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which facilitate the attachment of monocytes to vascular endothelium.[30,31] Circulating soluble forms of these adhesion molecules have been reported to be elevated in HD and PD patients[32,33] and are thought to reflect the extent of the inflammatory process and endothelial activation.[32] It has been conjectured that the modulation of adhesion molecules may therefore influence the progression of atherosclerosis.

AT is a potent lipid-soluble antioxidant that has been associated with a reduced risk of cardiovascular disease.[34-36] Supplementation with AT 400 to 1,200 IU/d in healthy subjects has been shown to protect LDL from in vitro oxidative modification.[37] In dyslipidemic individuals and hypercholesterolemic smokers, supplementation with AT decreased plasma lipid per-

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oxides and autoantibodies to OX-LDL and improved endothelial function.[38,39] Studies involving HD patients have also yielded promising results by demonstrating that AT supplementation increased LDL resistance to in vitro oxidative modification[7] and tended to improve LDL catabolism.[8] However, there is a paucity of data with regard to the effects of AT supplementation on LDL oxidation and endothelial function in chronic renal failure patients undergoing HD and PD. In a previous report, we have shown that AT supplementation decreases the oxidative susceptibility of LDL in chronic renal failure patients, with the effect being greater in PD patients.[40] In this communication, we tested the effect of native LDL and OX-LDL from chronic renal failure patients on HD and PD on monocyte endothelial adhesion and the levels of soluble CAMs and autoantibodies to OX-LDL in these patients. Also, we examined the effect of AT supplementation on these parameters.

SUBJECTS AND METHODS

Study Design

The study design was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center (Dallas, TX). Healthy subjects, HD patients, and PD patients were matched for age, gender, and body mass index (BMI), and studied in parallel sets. Sixteen HD patients, 17 PD patients, and 17 healthy controls (C) provided informed consent to participate in the study. Following a 10- to 12-hour fast, a blood sample was obtained from each participant at entry into the study. The subjects were then instructed to ingest one 400-IU capsule of RRR-AT with a meal 2 times daily for 12 weeks (ie, 800 IU/d). They were advised to maintain their usual diet and activities during the study and to report any side effects immediately to the investigators. A fasting blood sample was obtained again at the end of the supplementation period.

Study Population

Baseline characteristics of the study participants and a descriptive clinical summary of the dialysis patients are reported in Tables 1 and 2. The exclusion criteria for the study were (1) smoking, (2) use of antioxidant supplements (except for Nephrovite [R & D Labs, Marina Del Ray, CA], which contains vitamin B_1 1.5 mg, B_2 1.7 mg, B_6 10 mg, and B_{12} 6 mg, biotin 300 mg, nicotinamide 20 mg, pantothenic acid 10 mg, folate 1 mg, and vitamin C 60 mg), fish oil supplements, hypolipidemic medications, anticoagulant therapy, and thyroxine, (3) alcohol consumption greater than 1 oz/d, and (4) clinical evidence of coronary heart disease. The exclusion criteria were rigorous to minimize confounding factors. No more than one third of the dialysis patients had diabetes mellitus. All HD patients were on standard bicarbonate HD treatment (4 hours 3 times per week). All dialyzers were Baxter (Deerfield, IL) model CT190 with polysulfane membranes. PD patients were receiving either continuous ambulatory PD (2- to 2.5-L exchanges 4 times per day) or cyclic PD (20- to 24-L exchange for 10 to 12 hours) using dienyl membranes.

Table 1. Characteristics of C, HD, and PD Subjects

Characteristic	C (n = 17)	HD (n = 16)	PD (n = 17)
Age (yr)	39.4 ± 11.6	38.4 ± 11.2	37.2 ± 9.9
Sex ratio (male/female)	6/11	6/10	6/11
BMI (kg/m^2)	27.3 ± 5.8	24.0 ± 5.5	26.8 ± 5.9

NOTE. Values are the mean ± SD.

Table 2. Clinical Information for HD and PD Patients

Parameter	HD (n = 16)	PD (n = 17)
Diagnoses (n)		
Diabetes	1	1
Hypertension	5	8
Diabetes and hypertension	5	3
Glomerulonephritis	2	3
Pyelonephritis	—	1
Reflux nephropathy	1	—
Systemic lupus erythematosus	2	—
IgG nephropathy	—	1
Mean duration of dialysis (mo)	29.5	48
Laboratory values (mean ± SD)		
Serum creatinine (mg/dL)	12.1 ± 2.3	12.4 ± 4.3
Serum BUN (mg/dL)	60.2 ± 14.3	54.4 ± 16.1
Serum albumin (mg/dL)	4.0 ± 0.3	4.0 ± 0.3
Hematocrit (%)	33.3 ± 3.5	32.2 ± 5.4
Medications (n)		
Nephrovite	11	10
Erythropoietin	7	7
Beta-blockers	2	2
Estrogen	—	1
Insulin	1	4
Prednisone	1	—

Plasma Collection

Blood (60 mL) was obtained from subjects after a 10- to 12-hour fast at study entry and after 12 weeks of AT supplementation. Blood was drawn from HD patients immediately before their scheduled dialysis session. All blood samples were collected in tubes containing 1 mg/mL EDTA on ice. The plasma was separated by low-speed centrifugation at 4°C. Freshly separated plasma was used for determination of the lipoprotein profile and isolation of LDL. Aliquots were stored at −20°C for determination of plasma fatty acids, plasma AT, LDL AT, sICAM, sVCAM, sE-selectin, and autoantibodies to OX-LDL at the completion of the study.

Analytic Procedures

Plasma lipid and lipoprotein levels were measured using the Lipid Research Clinics methodology, except that cholesterol and triglycerides were determined enzymatically.[41] Plasma fatty acids were determined by gas-liquid chromatography after extraction and transmethylation,[42] with C17:0 as the internal standard (Nuchek-Prep, Elysian, MN). Plasma and LDL AT levels were measured by reverse-phase high-performance liquid chromatography following ethanol precipitation and hexane extraction.[43] The plasma concentration was expressed as micromolars and standardized to plasma lipid.[44] The protein concentration was determined by the method of Lowry et al.[45]

Lipoprotein Isolation and Oxidation

LDL isolation was performed by preparative ultracentrifugation at 4°C using a sterile NaBr-NaCl solution containing 1 mg/mL EDTA as previously described.[37,40] Immediately prior to use for oxidation and adhesion studies, LDL was dialyzed overnight in the dark against 1 L sterile phosphate-buffered saline (PBS), pH 7.4, at 4°C. To minimize endotoxin contamination, all solutions were made with endotoxin-free water (Baxter), all glassware used in the procedure was baked, LDL was sterile-filtered (0.22 μm), and plasma and LDL were handled under a sterile hood. We have previously reported on LDL oxidizability in these chronic renal failure subjects.[40] LDL was oxidized using 5 μmol/L Cu^{2+}, and oxidation was monitored by measurement of con-

jugated dienes and lipid peroxides as reported previously.[40] Oxidative modification of LDL was expressed as the susceptibility to oxidation (lag time), oxidation rate, and concentration of oxidative products in native and copper-oxidized LDL.[40]

Autoantibodies to OX-LDL

The relative concentrations of plasma autoantibodies to OX-LDL were determined using a modification of the method described by Uusitupa et al.[46] Briefly, pooled plasma from 8 healthy subjects was used to isolate LDL for antigen. Microplates were coated with 25 μg native or copper-oxidized LDL in PBS containing 0.27 mol/L EDTA and 20 μmol/L BHT, pH 7.4, and incubated overnight at 4°C. The plates were extensively washed with PBS containing 0.05% Tween 20 and with distilled water. They were then blocked with 2% human serum albumin (fatty acid–free; Sigma Chemical, St Louis, MO) and washed as previously described. Serum samples were diluted 1:50 using PBS buffer and 0.05% Tween 20, and 50 μL was dispensed in duplicate on the 2 microplates (ie, microplate containing native LDL antigen and microplate containing OX-LDL antigen). The plates were incubated overnight at 4°C and washed as previously described. Horseradish peroxidase–conjugated rabbit anti-human immunoglobulin G (Jackson Immunoresearch Laboratories, Westgrove, PA) was diluted 1:5,000 and added to the wells. The plates were then incubated for 4 hours at 4°C. The plates were washed and then incubated with peroxidase substrate, and the reaction was stopped by the addition of 2 mol/L H_2SO_4. Absorbance was measured at 492 nm, and the results were calculated as the ratio of OX-LDL to native LDL and OX-LDL–native LDL. The intraassay and interassay precision of this assay was less than 8%.

LDL-Induced Monocyte-Endothelial Adhesion

To study LDL-induced monocyte adhesion to vascular endothelium, we used an in vitro model/bioassay consisting of U937 cells, a cultured monocytic tumor cell line (American Type Culture Collection, Rockville, MD), and cultured human umbilical vein endothelial cells ([HUVECs] Clonetics Laboratories, San Diego, CA). These cell lines were maintained in 5% CO_2/95% air at 37°C. U937 cells were grown in suspension media RPMI 1640 containing 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine. HUVECs were grown in endothelial growth medium (EGM) as reported previously.[47] HUVECs were used 24 hours postconfluence between passages 3 and 10. The endotoxin concentration of HUVECs was tested by Clonetics and reported to be less than 0.125 EU/mL.

Freshly isolated LDL was dialyzed overnight in the dark against sterile PBS (pH 7.4). LDL (500 μg/mL) was incubated at 37°C for 5 hours in PBS with and without 25 μmol/L Cu^{2+}. The efficacy of the oxidation was measured by determining the concentration of conjugated dienes. After incubation, 1.25 mL native LDL and OX-LDL were passed through a 5-cm Chelex 100 column (BioRad, Richmond, CA) to remove the copper and were eluted with 1.75 mL sterile PBS. Lipopolysaccharide ([LPS] 10 ng/mL), native LDL (25 μg/mL), and OX-LDL (25 μg/mL) were added to confluent monolayers of cultured HUVECs and incubated in EGM for 2.5 hours at 37°C. After incubation, the supernatant was removed. U937 cells (0.5×10^6/mL) were coincubated with HUVECs in EGM for 30 minutes at 37°C and adhesion was performed as described previously.[47] Nonadherent cells were gently aspirated and washed with phenol red–free RPMI. Two hundred microliters of 0.125% Rose-Bengal in PBS was added to each well and incubated for 5 minutes at room temperature. The cells were washed twice with PBS containing 10% fetal calf serum. Eight hundred microliters of ethanol:PBS (1:1) were added and incubated for 30 minutes at room temperature. The absorbance of the ethanol extracts was measured at 570 nm.[47,48] Previously, we have shown that monocyte-endothelial adhesion as assessed by counting the cells per high-power field and absorbance from the Rose-Bengal assay yield similar data.[47] In previous dose-response experiments with LPS (1 ng/mL to 10 μg/mL), the addition of LPS (10 ng/mL) to endothelial cells resulted in a significant 2- to 2.5-fold increase in U937 adhesion compared with unstimulated endothelial cells. LPS for all experiments was from the same stock solution divided into aliquots and stored at −20°C. To reduce assay variability, the adhesion of U937 cells to HUVECs was expressed as a percentage of the LPS-induced adhesion after subtracting nonspecific binding for each parallel set of C, HD, and PD studied. The endotoxin level was measured in native LDL and OX-LDL samples by the Limulus Amoebocyte Lysate method (BioWhittaker, Walkersville, MD). The precautions used to minimize endotoxin contamination have already been described earlier. Only LDL samples containing less than 2 ng endotoxin/mg protein were used in this study. In previous studies, this level of endotoxin contamination was determined to result in less than 15% adhesion compared with 10 ng/mL LPS (data not shown).

Markers of Inflammation

Plasma sICAM and sVCAM were determined using sandwich enzyme-linked immunosorbent assay (ELISA) kits from Amersham (Arlington Heights, IL). The antibodies used are reported to be specific for sICAM and sVCAM and display less than 1% cross-reactivity with phospholipase A_2, interleukin-6 (IL-6), IL-1, tumor necrosis factor alpha, and sE-selectin. sE-selectin was determined in plasma samples using a monoclonal antibody ELISA kit from R&D Systems (Minneapolis, MN). This assay reportedly recognizes both recombinant and natural human sE-selectin, without any cross-reactivity with natural human IgG, recombinant human sVCAM-1, recombinant human sICAM-1, or natural human P-selectin. The absorbance for each assay was measured at 450 nm. The intraassay coefficient of variation of all 3 assays was less than 5%.

Statistics

Results are expressed as the mean ± SD. The number of subjects from whom samples were obtained is represented as "n." The Kruskal-Wallis nonparametric test was used to assess overall differences between the 3 groups. Baseline data and the response to supplementation (postsupplementation *v* baseline differences) were analyzed. Follow-up comparisons between C, HD, and PD were determined by Wilcoxon rank sum tests if the Kruskal-Wallis test was significant. Differences within groups were determined by Wilcoxon signed rank tests. Pairwise correlations between variables of interest were determined a priori, and Spearman rank correlation coefficients were used to assess these relationships. The level of significance (*P*) was set at .05 or less. Analyses were performed using SAS (SAS Institute, Cary, NC).

RESULTS

The characteristics of the study participants are shown in Tables 1 and 2. There were no significant differences in the age, gender distribution, or BMI between groups. The distribution of diabetes and hypertension was similar in the PD and HD groups, as was the use of erythropoietin, Nephrovite, and prednisone. As reported previously,[40] the HD and PD patients had significantly lower high-density lipoprotein cholesterol and moderately elevated triglycerides compared with the C subjects.

AT supplementation was tolerated well in all participants, with no changes in the blood chemistry profile, plasma lipids, or lipoproteins or adverse health reactions reported. Plasma fatty acids did not change during the course of the study, except for a slight but statistically significant increase in linoleic acid

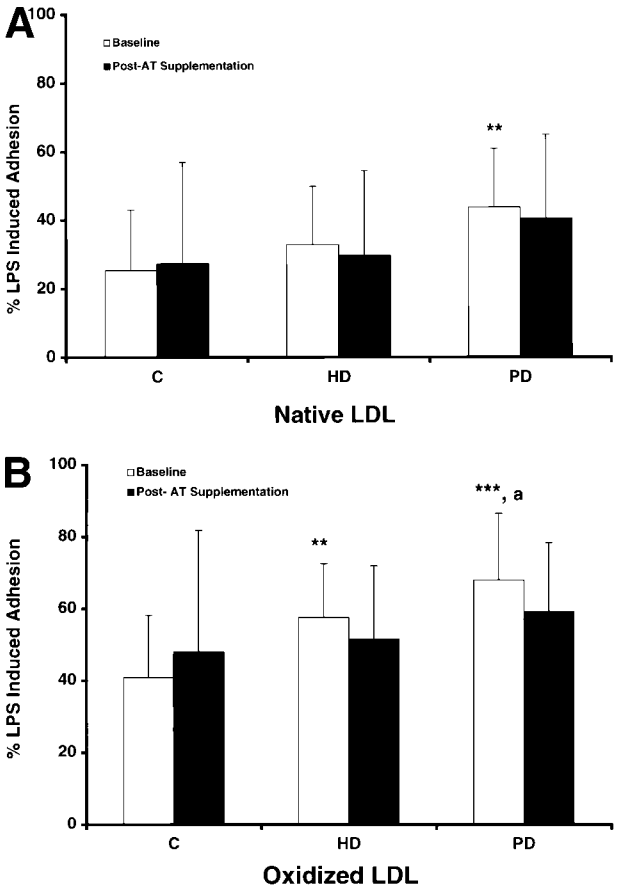

Fig 1. LDL-induced monocyte-endothelial cell adhesion. Results are expressed as the mean ± SD for the % adhesion of U937 cells to HUVECs relative to that induced by 10 ng/mL LPS. The Kruskal-Wallis nonparametric test and Wilcoxon rank sum test were used to determine statistical differences between groups. The Wilcoxon signed rank test was used to determine significant differences within a group. (A) Native LDL–induced adhesion is significantly higher at baseline in PD ($P \leq .01$) *v* C. AT supplementation had no significant effect (C, n = 16; HD, n = 16; PD, n = 14; paired data). (B) OX-LDL–induced adhesion is significantly higher at baseline in HD (**$P \leq .01$) and PD (***$P \leq .001$) *v* C; adhesion in PD ($^{a}P \leq .05$) is significantly higher *v* HD. AT supplementation had no significant effect (C, n = 15; HD, n = 14; PD, n = 14; paired data).**

in the C and PD groups.[40] Plasma lipid–standardized AT levels (μmol/mmol total lipid) increased significantly in all groups following supplementation (C, 3.06 ± 1.17 to 7.64 ± 2.46; HD, 2.95 ± 0.88 to 7.32 ± 2.86; PD, 3.56 ± 1.17 to 11.30 ± 9.27; $P < .005$). Similarly, there was a significant enrichment in LDL AT in all groups (C, 21.37 ± 10.11 to 41.64 ± 22.39 nmol/mg protein; HD, 17.89 ± 11.22 to 34.74 ± 24.07 nmol/mg protein; PD, 21.77 ± 10.50 to 51.20 ± 35.61 nmol/mg protein; $P < .005$). There were no differences in AT concentrations between groups before or after supplementation.

Native LDL– and OX-LDL–induced monocyte-endothelial cell adhesion relative to the LPS agonist at baseline are shown in Fig 1A and B, respectively. Native LDL from PD patients stimulated significantly more adhesion compared with LDL from C subjects ($P = .0028$). OX-LDL from HD and PD patients promoted significantly more adhesion than OX-LDL from C subjects ($P < .01$ and $P < .001$, respectively). Furthermore, significantly more adhesion was stimulated by OX-LDL from PD patients compared with HD patients ($P = .03$). AT supplementation did not effect adhesion of monocytes to endothelial cells induced by native LDL. OX-LDL–induced adhesion tended to decrease after HD and PD patients received AT supplementation, but this was not statistically significant. Endotoxin contamination of LDL was minimal in the study and maintained at less than 2 ng/mg protein. There were no significant differences in the endotoxin levels of LDL among the 3 groups (0.29 ± 0.55, 0.22 ± 0.47, and 0.11 ± 0.21 ng/mg LDL in C, HD, and PD groups, respectively).

No significant differences were observed in the amount of lipid peroxides in native LDL in the 3 groups. Nor were there significant differences between groups for the maximum concentration of conjugated dienes and lipid peroxides in OX-LDL (Table 3). AT supplementation did not have a significant effect on lipid peroxides in native LDL or the maximum amount of conjugated dienes and lipid peroxides in OX-LDL.

Since chronic renal failure patients have elevated levels of advanced glycated end products and this may be a possible mechanism to explain the greater monocyte adhesion in the dialysis patients,[49-51] plasma levels of methylglyoxal were measured in frozen plasma from a subset of patients (n = 8). Methylglyoxal levels were quantified by gas chromatography/mass spectrometry in the laboratory of P.J. Beisswenger, MD.[52] Plasma levels of methylglyoxal were significantly higher in HD and PD patients compared with controls (HD, 1,014 ± 397; PD, 582 ± 155; C, 98 ± 38 nmol/L; $P < .001$). Furthermore, levels were significantly higher in the HD group versus the PD group ($P = .006$).

Plasma levels of sVCAM-1, sE-selectin, and sICAM-1 are shown in Fig 2. Plasma sVCAM-1 was approximately 2 to 3 times higher ($P < .001$) in HD and PD subjects compared with the C group. Patients on HD had significantly higher plasma sVCAM-1 than those on PD ($P = .05$). Plasma sE-selectin was also elevated in HD and PD patients compared with C subjects ($P < .01$ and $P < .001$, respectively). Plasma sICAM-1 was not significantly different in dialysis patients compared with C subjects. Supplementation with AT had no effect on the plasma concentration of soluble CAMs.

Plasma titers of autoantibodies to OX-LDL are shown in Table 4. The relative concentration of autoantibodies are expressed as the ratio between absorbance values obtained when plasma samples were added to microplates coated with OX-LDL antigen versus microplates coated with native LDL antigen. The relative concentrations of autoantibody titers were similar in the C, HD, and PD groups at baseline. We previously

Table 3. Measures of Lipid Peroxidation in Native LDL and OX-LDL (nmol/mg protein)

Parameter	C (n = 17)	HD (n = 14)	PD (n = 15)
Native LDL lipid peroxides	267 ± 113	281 ± 136	270 ± 131
OX-LDL conjugated dienes	394 ± 69	394 ± 92	382 ± 50
OX-LDL lipid peroxides	569 ± 105	549 ± 140	553 ± 112

NOTE. Values are the mean ± SD.

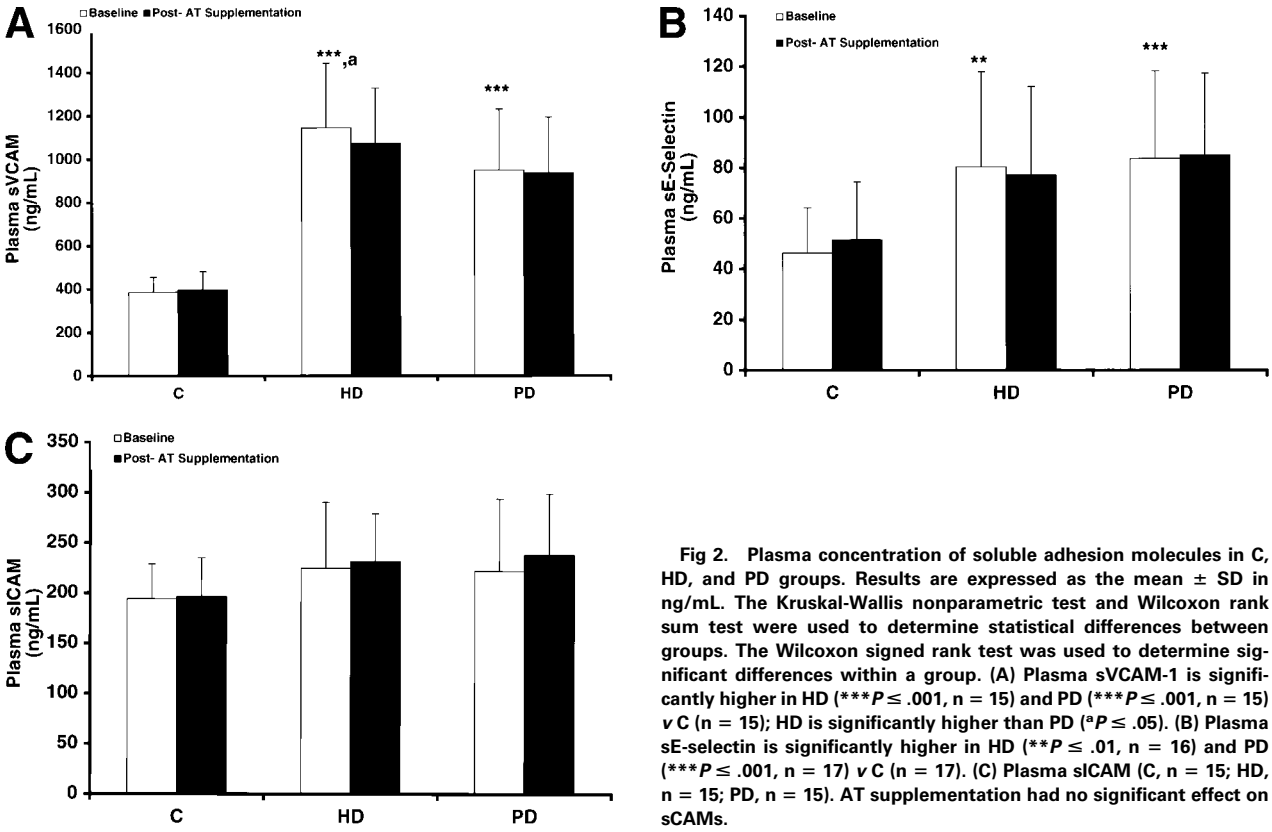

Fig 2. Plasma concentration of soluble adhesion molecules in C, HD, and PD groups. Results are expressed as the mean ± SD in ng/mL. The Kruskal-Wallis nonparametric test and Wilcoxon rank sum test were used to determine statistical differences between groups. The Wilcoxon signed rank test was used to determine significant differences within a group. (A) Plasma sVCAM-1 is significantly higher in HD (*$P \leq .001$, n = 15) and PD (***$P \leq .001$, n = 15) *v* C (n = 15); HD is significantly higher than PD ($^{a}P \leq .05$). (B) Plasma sE-selectin is significantly higher in HD (**$P \leq .01$, n = 16) and PD (***$P \leq .001$, n = 17) *v* C (n = 17). (C) Plasma sICAM (C, n = 15; HD, n = 15; PD, n = 15). AT supplementation had no significant effect on sCAMs.**

reported[40] that AT supplementation prolonged the lag phase of LDL oxidation in these healthy subjects and chronic renal failure patients. However, supplementation with AT failed to decrease autoantibodies to OX-LDL.

There was a significant correlation between the baseline conjugated-diene lag time and native LDL–induced monocyte-endothelial adhesion in C subjects ($r = -.54$, $P = .02$) and OX-LDL–induced adhesion in PD patients ($r = -.62$, $P = .01$). No associations were observed between LDL oxidative indices and adhesion in HD patients or when HD and PD patients were pooled.

DISCUSSION

Patients with chronic renal failure have accelerated atherosclerosis. OX-LDL stimulates adhesion molecule expression,[53,54] promotes interaction between monocytes and endothelial cells,[29,53,54] and leads to atherosclerotic plaque formation.[26-29,53] Increased oxidative modification of LDL has been reported in patients receiving hemodialysis treatment[5-7] but not in patients undergoing PD.[11] In a previous report, we failed to demonstrate increased oxidative susceptibility of LDL in these chronic renal failure patients.[40] In accordance with these findings, we also failed to show elevated titers of antibodies to OX-LDL in the present study. To date, only one group has reported elevated autoantibodies to OX-LDL in dialysis patients, but these patients also had LDL with increased oxidative susceptibility relative to matched controls.[5,6] Control and dialysis patients in our study had similar degrees of LDL oxidation. The difference in our findings could be due to the fact that the dialysis patients in our study had normal plasma and LDL AT and elevated plasma oleic acid,[40] were relatively young, and were free of clinical symptoms of coronary heart disease. Autoantibodies to OX-LDL have been shown to correlate with coronary artery disease (CAD)[55-57] and may be clinically relevant markers of cardiovascular risk, although some research has failed to corroborate the link.[46,58] The method we used to measure plasma titers is based on a previously published technique that demonstrated significant correlations between autoantibody titers to OX-LDL and atherosclerosis.[59] Since these HD and PD patients were free of

Table 4. Autoantibodies to OX-LDL in Dialysis Patients and Matched Controls

Parameter	C Subjects		HD Patients		PD Patients	
	Baseline	Post-AT	Baseline	Post-AT	Baseline	Post-AT
OX-LDL/N-LDL ratio	1.59 ± 0.20	1.61 ± 0.29	1.64 ± 0.38	1.62 ± 0.30	1.67 ± 0.36	1.77 ± 0.36
OX-LDL − N-LDL	0.22 ± 0.07	0.21 ± 0.06	0.22 ± 0.1	0.23 ± 0.07	0.23 ± 0.09	0.24 ± 0.09

NOTE. Values are the mean ± SD.

heart disease and the oxidative profile of their LDL did not differ versus the C group, it is not surprising that they also had normal titers of autoantibodies to OX-LDL.

A novel observation in this report is that native LDL from PD patients and OX-LDL from both PD and HD patients stimulated a greater adhesion of monocytes to endothelium compared with control LDL, and thus is more atherogenic. While the increased adhesion was 1.4- to 1.7-fold, it should be viewed in the context that atherogenesis occurs over several decades. Since the endotoxin levels were similar between the 3 groups, endotoxin contamination cannot explain the increased adhesion observed. Also, lipid peroxidation of LDL cannot solely explain the differences in LDL-induced adhesion among the C, HD, and PD groups, since the AT, conjugated diene, and lipid peroxide contents of the LDL were similar. Furthermore, no significant correlations associated the endogenous levels of LDL oxidation with native LDL–induced monocyte adhesion to endothelial cells. Only oxidative susceptibility of LDL correlated with OX-LDL–induced adhesion in the PD group. Some other component or modification of the LDL particle may be responsible for the greater adhesion stimulated by LDL from HD and PD patients. Alterations in the lipid and apolipoprotein content of LDL have been reported in chronic renal failure patients.[10,16-19] Lipid-rich LDL containing a high ratio of cholesterol and triglyceride to protein has been shown to enhance native LDL–induced adhesion.[60] Sutherland et al[10] reported higher amounts of triglyceride in LDL from dialysis patients and less protein in LDL from PD patients compared with HD patients. The lipid and lipoprotein profiles of HD and PD patients in our study are consistent with these compositional differences.[40] LDL from chronic renal failure patients may also be modified by elevated plasma homocysteine.[61-63] Homocysteine has been shown to promote monocyte adhesion to endothelial cells and cause endothelial dysfunction and activation.[64,65] Preliminary studies on a subset of dialysis patients participating in this study revealed hyperhomocystinemia in 7 of 7 HD subjects (34.8 ± 12.4 μmol/L) and 5 of 6 PD subjects (23.6 ± 10.0 μmol/L). However, we found no significant correlations between plasma homocysteine and LDL-induced monocyte-endothelial cell adhesion in these small patient subsets. Additionally, nonenzymatic glycation of LDL may occur in chronic renal failure patients.[22,24] Glycated LDL is readily oxidized and has been found to impair endothelial function more potently than OX-LDL.[66] Advanced glycation end products have been shown to enhance the expression of VCAM-1, upregulate ICAM-1 and E-selectin, and increase endothelial cell adhesion.[67] In a subgroup of patients, we showed significantly higher levels of methylglyoxal, a measure of the advanced glycation of proteins. However, while the LDL from PD patients promoted more adhesion, methylglyoxal levels were significantly higher in the plasma of HD patients. While this finding would suggest that advanced glycation of LDL is unlikely to be the major mechanism accounting for the increased level of monocyte-endothelial cell adhesion in PD patients, it should be emphasized that plasma and not LDL methylglyoxal levels were measured. Future studies will focus on elucidating the effects of LDL composition and modification on the stimulation of monocyte-endothelial cell adhesion in the milieu of chronic renal failure.

Chronic renal failure is thought to be an inflammatory disorder. Elevated cytokines, typically released in inflammatory processes, induce the expression of adhesion molecules in various cells.[31,72] ICAM-1 is expressed by a wide variety of cells.[31,32,68] VCAM-1 is expressed by macrophages, lymphoid dendritic cells, and endothelial and epithelial cells, and E-selectin is expressed only by activated vascular endothelium.[31,32,68] Both sE-selectin and sVCAM-1 are therefore fairly specific markers of endothelial activation. In our study, plasma sE-selectin and sVCAM-1 were elevated in dialysis patients, in agreement with the literature,[32,33,69] and HD patients had significantly greater sVCAM-1 than PD patients. There was a trend for elevated sICAM-1 in dialysis patients, but this was not significant. Elevated sICAM-1 levels have been observed in dialysis patients,[32,33] but plasma levels have also been reported to be only slightly higher in HD subjects compared with normal subjects.[70] Burkhardt et al[71] found that there may be a diffuse increase in sICAM-1 in conditions such as glomerulonephritis and lupus nephritis, and normal or even reduced levels in conditions such as membranous nephropathy. Therefore, the results we obtained in this study are reasonable. The adhesion molecule profile for HD and PD patients in our study is consistent with endothelial activation, and this is further confirmed by the fact that plasma sVCAM-1 levels in PD and HD patients were similar to those reported in essential hypertensive patients with peripheral vascular disease.[72] Furthermore, HD and PD patients have been reported previously to have endothelial dysfunction manifested by impaired endothelium-dependent vasodilation.[73,74] We found no significant correlations between adhesion molecules and creatinine, suggesting that the degree of renal failure per se was not the underlying cause of elevated plasma adhesion molecules. Instead, circulating LDL lipid peroxides in HD patients and plasma homocysteine in PD and HD patients correlated with sE-selectin and sVCAM-1. Since OX-LDL and hyperhomocystinemia are known to cause endothelial cell dysfunction,[28,64,65] these factors may contribute to the apparent endothelial dysfunction and activation in dialysis patients. Also, the elevated advanced glycation end-product levels may contribute to the endothelial dysfunction. Additionally, established cardiovascular risk factors such as diabetes, hypertension, and dyslipidemia are also likely to be responsible for promoting endothelial dysfunction in this population.

AT is a potent antioxidant, and supplementation with this vitamin has been shown to increase LDL resistance to oxidation and to have significant effects on cellular function, including monocyte-endothelial adhesion.[47] Since we previously demonstrated that supplementation of these patients with AT 800 IU/d increases the oxidative resistance of LDL,[40] we also anticipated significant effects on LDL/OX-LDL–induced monocyte-endothelial adhesion and soluble CAMs. However, this dose of AT failed to significantly alter LDL/OX-LDL–induced monocyte-endothelial adhesion and soluble CAMs. These results may be explained by the fact that markers of LDL oxidation were not elevated in the C, HD, and PD groups, and it would therefore be unlikely that supplementation with AT would further reduce the level of LDL oxidation in order to have a significant effect on cell function. This is consistent with the findings of a recent study in which AT 544 IU/d had no effect on endothelial function in clinical populations with nor-

mal levels of autoantibody titers to OX-LDL, but in patients with elevated titers, supplementation reduced autoantibody levels and improved endothelial function.[39] This suggests that the beneficial effects of AT supplementation on endothelial function may be limited to conditions in which excessive oxidative stress and LDL oxidation can be attenuated by the antioxidant. This is also illustrated by the findings of Cominacini et al[53] in which in vitro AT enrichment of LDL in vitro prior to oxidation significantly reduced LDL conjugated-diene production and LDL-induced monocyte-endothelial cell adhesion. In our adhesion study, in vivo AT supplementation did not alter endogenous lipid peroxides in native LDL or protect LDL from maximum oxidation. Since maximal oxidation of LDL was obtained, the AT content of OX-LDL was depleted regardless of prior enrichment. As a result, AT supplementation did not change the concentration of oxidation products in OX-LDL or the subsequent amount of LDL-induced adhesion. It is possible that if we had oxidized LDL for a shorter period, the trend to reduced adhesion would be significant, since we showed a significant prolongation of the lag phase in these patients.[40] In other supplementation studies, AT 1,200 IU/d in healthy[47] or diabetic[75] subjects has been shown to reduce the adhesion of enriched monocytes to cultured endothelial cells. However, incubating AT-enriched native LDL or OX-LDL with endothelial cells may not inhibit the expression of adhesion molecules or monocyte-endothelial adhesion as effectively as direct AT enrichment of endothelial cells[76] or monocytes.[47,75] Future studies will be directed at investigating the effect of AT supplementation on monocyte function in renal failure patients. Although in vivo supplementation of diabetic patients with AT 1,200 IU/d appears sufficient to decrease cytokine release from monocytes and reduce sICAM-1, sVCAM-1, and sE-selectin,[75] it is not clear if AT 800 IU/d would also have these effects. Since dialysis patients in our study had significantly elevated levels of adhesion molecules and multiple risk factors for CAD, it is probable that doses of AT in excess of 800 IU/d would be necessary to overcome the various deleterious inflammatory processes affecting the vascular endothelial function. There is a paucity of data on the effect of AT on sCAMs or cell function in chronic renal failure patients, and this is an area that needs to be pursued.

This is the first study to comprehensively report on LDL oxidation, CAMs, and monocyte-endothelial cell adhesion in chronic renal failure patients receiving HD or PD treatment. LDL from patients with chronic renal failure appears significantly more atherogenic than LDL from healthy subjects, since monocyte-endothelial cell adhesion was enhanced by incubation with native LDL and OX-LDL from these subjects. This stimulatory effect is more pronounced in LDL from PD patients, particularly under conditions of oxidative stress. The exact component of LDL responsible for promoting adhesion is currently unknown. HD and PD patients also have significantly elevated sVCAM-1 and sE-selectin, indicative of endothelial activation. Supplementation with AT 800 IU/d was not effective in decreasing LDL-induced monocyte-endothelial adhesion or sVCAM-1 or sE-selectin levels. Further studies will be directed at elucidating the moiety in LDL that promotes monocyte-endothelial adhesion and determining if supplementation with higher doses of AT will have a benefit on these vascular parameters in patients with chronic renal failure.

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