Serum Vitamin E and Oxidative Protein Modification in Hemodialysis: A Randomized Clinical Trial

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Background: Patients with end-stage renal disease have increased circulating concentrations of oxidatively modified circulating proteins. Therefore, we examined the ability of vitamin E α (α-tocopherol) to alter levels of these modified proteins.

Study Design: Randomized clinical trial.

Setting & Participants: 27 clinically stable patients treated by means of hemodialysis in 4 freestanding outpatient dialysis units.

Intervention: Oral administration of 800 IU of vitamin E α or placebo daily.

Outcomes & Measurements: Plasma levels of α- and γ-tocopherol and oxidative protein modifications reflecting 2 pathways for protein-oxidant damage. The advanced glycation end product pentosidine reflects glycoxidation. The lipid peroxidation products iso[4]-levuglandin E_2, (E)-4-hydroxy-2-nonenal, and (E)-4-oxo-2-nonenal are formed through covalent adduction.

Results: Circulating levels of all oxidative protein modifications were increased in patients with end-stage renal disease. Supplementation with α-tocopherol caused α-tocopherol levels to rise (13.2 $\pm$ 3.7 to 27.3 $\pm$ 14 μg/mL), but γ-tocopherol levels to decrease (4.1 $\pm$ 1.6 to 3.5 $\pm$ 1.1 μg/mL). Control values were unchanged. There was no effect on oxidative protein modifications (placebo versus treatment; mean for pentosidine, 15.6 $\pm$ 11.4 (SD): 95% confidence interval (CI), 8.2 to 23.1 versus 21.3 $\pm$ 9.0 pg/mg protein; 95% CI, 16.1 to 26.6; iso[4]-levuglandin E_2, 8.31 $\pm$ 2.55; 95% CI, 6.77 to 9.85 versus 8.46 $\pm$ 2.37 nmol/mL; 95% CI, 7.09 to 9.84; (E)-4-hydroxy-2-nonenal, 0.51 $\pm$ 0.11; 95% CI, 0.45 to 0.57 versus 0.51 $\pm$ 0.08 nmol/mL; 95% CI, 0.46 to 0.56; (E)-4-oxo-2-nonenal, 189 $\pm$ 44; 95% CI, 162 to 215 vs 227 $\pm$ 72 pmol/mL; 95% CI, 183 to 271).

Limitations: Sample size was adequate to show changes in α- and γ-tocopherol levels in response to treatment. However, power was insufficient to show an effect on oxidative protein modifications.

Conclusions: Intervention of oral supplementation with α-tocopherol did not result in changes in circulating oxidative protein modifications. A larger study may be required to show an effect in this clinical setting.

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INDEX WORDS: Vitamin E; α-tocopherol; γ-tocopherol; pentosidine; isolevuglandin; hydroxynonenal; oxononenal; hemodialysis; end-stage renal disease.

In the early 1990s, epidemiological studies established an inverse relationship between supplemental vitamin E use and cardiovascular disease.[1] By 2000, an estimated 23 million Americans used supplemental vitamin E. However, large prospective randomized controlled trials of treatment failed to show improved cardiovascular or other health outcomes.[2,3] Thus, proponents of antioxidant therapy argued that greater benefit might be found in the setting of abnormal levels of oxidative stress, such as in patients with end-stage renal disease (ESRD) treated with dialysis.[4]

Excess oxidant production and decreased antioxidant defense[5] have been recognized characteristics of the uremic state. In patients with ESRD, oral vitamin E supplementation decreased the susceptibility of low-density lipoprotein (LDL) to oxidation[6] and prevented the oxidative stress associated with intravenous iron administration for treatment of patients with anemia.[7] The Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease trial (SPACE) showed a significant decrease in cardiovascular events in 196 dialysis patients with preexisting cardiovascular disease administered oral vitamin E supplements.[8] These results emphasized the importance of understanding the basic mechanisms of action of antioxidants in the uremic milieu.

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Clinical observation that vitamin E was protective against morbid events in some,[8,9] but not in all, settings was named the antioxidant paradox.[4] Factors that lead to oxidative stress in living systems are complex. For example, the kinetics of reaction between vitamin E and oxidants is slower than the rate at which free radical species inactivate nitric oxide.[10] Therefore, vitamin E would not be expected to correct abnormalities in flow-mediated vasodilation[11] despite effectively decreasing LDL oxidation.[6] In addition, vitamin E was shown to act as a pro-oxidant, particularly in the absence of vitamin C.[12]

Patients with ESRD have increased quantities of oxidatively modified circulating and tissue proteins, including advanced glycation end products (reflecting both oxidative and carbonyl stress),[13] advanced oxidative protein products, oxidized LDL, (E)-4-hydroxy-2-nonenal (hydroxynonenol, HNE)–derived-2-pentylpyrroles, and isolevuglandin adducts,[14,15] as well as oxidized lipids, including isoprostanes[16,17] and oxysterols.[17] The goal of this project was to study the effect of supplemental vitamin E on levels of oxidative protein modifications. We measured pentosidine, a glycoxidation-derived protein modification, as well as protein modifications derived from the lipid peroxidation products iso[4]-levuglandin E_2 (iso[4]-LGE_2), HNE, and (E)-4-oxo-2-nonenal (ONE) in a highly oxidative clinical state—ESRD treated by means of hemodialysis. We explored relationships between levels of these products at baseline and during the course of 6 months of treatment to develop hypotheses about the relationship between levels of oxidation products and factors known to influence oxidation in the clinical setting.

METHODS

Patients

At the time this study was initiated, 286 patients with ESRD were treated by means of hemodialysis at Centers for Dialysis Care Cleveland, Ohio, under the care of nephrologists at University Hospitals of Cleveland. Charts were reviewed from 168 of these patients recommended by the primary dialysis nurse to be clinically stable. Inclusion criteria included uninfected patients with an upper-extremity polytetrafluoroethylene graft or arteriovenous fistula. Exclusion criteria were current use of vitamin E or anticoagulant therapy, lower-extremity vascular access, central venous catheter access, central venous stenosis or clotting disorders, and use of anticoagulants other than heparin during hemodialysis. One hundred thirty-four patients were considered ineligible or declined to participate. Informed consent was obtained in compliance with the Institutional Review Board of University Hospitals of Cleveland from 34 patients. Patients were recruited between June 2002 and September 2003, with study closeout in March 2004. During that time, all patients received treatment using polysulfone high-flux dialyzers (F80; Fresenius, Walnut Creek, CA, or APS1050; Asahi, Tokyo, Japan). Patients left the study because of death, transplantation, or initiation of anticoagulant therapy.

Study Design

Patients were assigned to treatment based on a random-number-generation protocol performed by the hospital pharmacy at the time of dispensing treatment or placebo. Participants were enrolled by the study investigators and assigned to treatment or placebo by the dispensing hospital pharmacy. Investigators and patients were blinded to treatment with vitamin E (α-tocopherol, 800 IU) or identical placebo capsules to be administered daily until the end of the study. "Natural vitamin E" (RRR-α-tocopherol, also known as d-α-tocopherol) was the kind gift of Cognis Inc (LaGrange, IL). Clinical data were collected by means of interview and chart review. Clinical parameters and routine clinical chemistry and hematology testing were performed in a single reference clinical laboratory. Results were recorded by using chart review every 3 months throughout the study. Total administered doses of erythropoietin and intravenous iron were tallied at each 3-month period, as were significant clinical events, including hospitalizations and surgical interventions.

Assays

At each time point, blood was collected in EDTA-metal–free tubes for plasma or plain glass tubes for serum. Samples for plasma were placed on ice and spun in the cold at 3,000 rpm for 15 minutes. Serum was allowed to clot before centrifugation. Plasma for analysis of α- and γ-tocopherol was protected from light at each step. Plasma for enzyme-linked immunosorbent assay was stabilized before storage by the addition of butylated hydroxytoluene (10 μmol/L final concentration) and protease inhibitors, as described.[14] After processing, serum and plasma aliquots were quench-frozen in liquid nitrogen and transported to the laboratory, where they were layered with argon and stored at -80°C.

Tocopherols were measured using a modification of the method of Sommerburg et al.[18] Briefly, plasma was extracted using ethanol and hexane, dried under nitrogen, and reconstituted in methanol before high-performance liquid chromatography with a C18 column and UV detection at 292 nm. Standards for α- and γ-tocopherol were purchased from Sigma (St Louis, MO) and prepared freshly for each assay. Intra-assay coefficients of variation were 0.039 for α-tocopherol and 0.041 for γ-tocopherol, and interassay coefficients were 0.078. The normal range for α-tocopherol was 12.50 ± 0.23 μg/mL, and that for γ-tocopherol was 2.41 ± 0.26 μg/mL.

Pentosidine was measured by means of high-performance liquid chromatography using a modification of the