Antioxidant Therapy with Vitamin E for the Reduction of sVCAM-1 and sICAM-1 Levels in Diabetics with Microalbuminuria

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A. Study Purpose and Rationale

Considerable investigation has been directed at identifying the exact mechanism by which the abnormalities of glucose homeostasis seen in diabetes mellitus lead to the disease's serious long-term sequelae. Coronary artery disease, stroke, blindness, and renal failure are the end result of vascular damage, and account for most of the morbidity and mortality of diabetes. Knowledge of the link between hyperglycemia and microvascular injury may provide opportunities for intervention in the disease process.

Recent evidence has implicated the glycation of proteins as an important step in this process. Glucose reacts nonenzymatically with a free amino group, with subsequent oxidation-reduction reactions and molecular rearrangements leading ultimately to the formation of advanced glycation endproducts, (AGEs).1,2,3 AGE's may then mediate diabetic vascular injury in a number of ways. Glycation promotes protein crosslinking which can alter the structure and function of extracellular matrix proteins.3,4 Intracellular glycation may post-translationally modify proteins and thus interfere with metabolic pathways.5 AGE's have also been shown to interact with specific cellular receptors6 inducing the expression of specific cell adhesion molecules by an oxidant dependent mechanism.7 The generation of free radicals (oxidant stress) is felt to be a critical step in the process of vascular injury.

The expression of two cell adhesion molecules, VCAM-1 and ICAM-1, is enhanced via AGE/AGE-receptor interaction.8 Thus elevated VCAM-1 or ICAM-1 may serve as a marker for AGE mediated free radical damage. Other evidence suggests that VCAM-1 may play a more direct role in vascular endothelial cell damage, possibly binding monocytes and lymphocytes in areas of atherosclerotic plaque formation.9

VCAM-1, ICAM-1, and other cell surface adhesins are released in measurable quantities from endothelial cells in both normals and in disease states by an unknown mechanism.10 The soluble forms of these two molecules, sVCAM1 and sICAM-1, have been shown to be significantly elevated in diabetic patients with microalbuminuria.11 These observations have led investigators to hypothesize that a reduction in oxidant stress in these patients, using antioxidant therapy with Vitamin E, might reduce or suppress AGE effects. Vitmain E has been shown to decrease glycosylated hemoglobin levels in diabetic patients after only one month of therapy.12 Efforts are now being directed at assessing the effects of Vitamin E on later steps in the pathway, investigating whether suppression of AGE effects and subsequent VCAM/ICAM expression may mitigate AGE-mediated vascular injury.

A recent pilot study was performed at CPMC to test this hypothesis. Nine patients with diabetes and microalbuminuria, all with elevated baseline levels of sVCAM-1 and sICAM-1, were treated with 800 IU of Vitamin E for 9 weeks. sVCAM-1 levels decreased an average of 14% ($p<0.05$), while sICAM levels decreased an average of 8% ($p<0.05$) (Smith, et al., unpublished data). This study proposes to further test the hypothesis that Vitamin E can reduce VCAM and ICAM levels and ultimately slow the progression of albuminuria and diabetic renal disease.

B. Study Design and Statistical Analysis

a. Design

This is to be a double-blinded, placebo controlled trial comparing the effect of Vitamin E to that of placebo on serum levels of VCAM-1 and ICAM-1 and on microalbuminuria in 40 patients with

diabetes and early diabetic nephropathy. Subjects will be recruited from the Associates of Internal Medicine Practice and from Renal Clinic at Columbia Presbyterian Medical Center (CPMQ. Subjects will also be identified by searches of the CPMC laboratory results computer database to identify patients with 24 hr. urine collections positive for microalbuminuria. Patients will be interviewed in an initial visit to determine their eligibility for the study (see Study Subjects below for details).

Eligible subjects will return for a subsequent visit (Day 0) prior to randomization to obtain informed consent, for phlebotomy to obtain baseline BUN, Creatinine, VCAM-1, ICAM-1, and Vitamin E levels, and to arrange for two overnight urine collections to determine baseline microalbuminuria. Subjects will then be randomly assigned to either the Treatment Group or the Control Group. A prolonged run-in period will be utilized; subjects randomized to both groups will begin, on day 0, daily oral doses of placebo. Phlebotomy for VCAM-1, ICAM-1, Vitamin E level, and Creatinine will be repeated on Day 14, and the average of values from Day 0 and Day 14 will be used to establish baseline values for subjects in both groups. Studies have shown that VCAM and ICAM levels in untreated individuals can vary up to 40%; we plan to use an average of two values for VCAM and ICAM levels to determine baseline and end-study levels to minimize error introduced by this variability.

On Day 14, subjects in the Treatment Group will then change from placebo to Vitamin E 800 IU daily, while those in the control group will continue daily placebo. Placebo and Vitamin E will be prepared by the Research Pharmacy in identical opaque gelatin capsules. Phlebotomy will then be repeated every two weeks in all subjects for a total of 16 weeks. Values from Day 98 and Day 112 will be averaged to determine levels at the completion of the study. End-study levels of microalbuminuria. will be measured as the average of two overnight urine collections for protein performed between Day 98 and 112.

b. Schedule

Day	Dosing		Testing
	Treatment Group	Control	
0	placebo	Placebo	Vit E, sVCAM, sICAM-1, Chem 7 Overnight protein (2)
14	Vit E 800IU	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
28	Vit E 800IU	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
42	Vit E 8001U	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
56	Vit E 8001U	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
70	Vit E 8001U	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
84	Vit E 8001U	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
98	Vit E 8001U	placebo	Vit E, sVCAM-1, sICAM-1 Chem 7
112	Vit E 800IU	palcebo	Vit E, sVCAM, sICAM-1, Chem 7 Overnight protein (2)

c. Randomization

Prior to the start of the study, 40 separate treatment regimens, 20 Placebo Group and 20 Treatment Group, will be prepared by the Research Pharmacy and randomized to a treatment number (1-40). A fixed randomization scheme will be utilized, with an allocation ratio of 1:1. Given the potential

for an extended period of patient recruitment and enrollment, nonuniform blocked allocations will be made. Investigators enrolling patients into the study will assign treatment numbers sequentially 1 to 40 and maintain documentation of each subject's name, CPMC medical record number, and treatment assignment. Records documenting the coding of treatment assignments will be kept by the Research Pharmacy and will be available to the investigator at the termination of the study.

d. Power calculations

The pilot study showed a mean 14 % decrease in sVCAM-1 levels for 9 subjects, with a standard deviation of 7.8%. Using standard power calculations, a study involving 16 subjects should have sufficient power to demonstrate a statistically significant difference between treatment and control groups. 40 subjects will be enrolled in the proposed study.

e. Analysis

The results of a recently completed, uncontrolled pilot study involving nine treated subjects demonstrated a statistically significant difference in baseline and end-study mean levels of VCAM and ICAM levels. Significant variability existed, however, among the treated subjects. For this reason, data for each subject will be reported as a % change from baseline, and mean values for ICAM-1 and VCAM-l for Treatment and Control Groups will be reported as a mean % change from baseline. Statistical analysis will be performed using a paired student's t-test.

C. Study Procedures

As outlined above, study subjects will be required to submit to phlebotomy on enrollment and biweekly thereafter for 14 weeks. This will require biweekly visits to CPMC, each lasting approximately 15-30 minutes. Phlebotomy will consist of the removal of approximately 10 cc of blood from a peripheral vein under sterile conditions. Four overnight urine collections will be performed by the subject at home at the specified time. Subjects will void prior to bedtime, then collect all urine voided in the course of the night and the first AM voiding in a plastic container. Collected urine will then be brought by the subject to CPMC the following day.

Each subject will participate in the study for a period of 14 weeks. The entire study should be completed in approximately one year from enrollment on the first subject.

D. Study Drugs

Subjects in the treatment group will receive a single daily oral dose of 800 international units of Vitamin E for the duration of the study. The Vitamin E will be given in the alpha tocopherol form, the most biologically active compound in the family of tocopherols and tocotrieneols. No adverse effects of Vitamin E at this dose and duration of therapy have been identified. A comprehensive review of the literature from 1986 to 1992 [13] revealed 27 studies using doses up to 2400 IU of Vitamin E for periods up to 4.5 years, with no report of adverse effect. Meydani, et al., in a placebo-controlled, double-blinded study of 800 IU of Vitamin E in healthy adults over age 60, showed no effect on hepatic function, renal function, thyroid function, and various hematological parameters. [14]

Vitamin E at high doses (4000 IU) has been shown to have a poorly characterized antiplatelet effect that can exacerbate the bleeding tendency of patients receiving Warfarin for anticoagulation. Patients on Warfarin will be excluded from the study.

Patients in the control group (and patients in the treatment group for Day 0 through Day 14) will be given daily doses of a placebo preparation, containing pharmaceutical grade mineral oil in an opaque gelatin capsule identical to the Vitamin E preparation. Both placebo and Vitamin E will be prepared by the research pharmacy and placed in containers for each study subject labeled with his or her assignment number.

E. Medical Devices

Not applicable

F. Study Subjects and Recruitment Study

Sbjects will be recruited from the Associates of Internal Medicine Practice and from Renal Clinic at Columbia Presbyterian Medical Center (CPMQ- Subjects will also be identified by searches of the CPMC laboratory results computer database to identify patients with 24 hr. urine collections positive for microalburninuria. No restrictions will be made based on gender or race.

a. Inclusion criteria

1. Age > 18 years
2. Diabetes mellitus (type I or type II) diagnosed using standard criteria
3. Urinary albumin excretion rates between 30 and 300 mg/24 hours
4. sVCAM-l levels greater than or equal to 7OOng/ml, as measured by enzyme linked immunoabsorbent assay
5. Stable glycemic control on either oral hypoglycemic or insulin therapy, as indicated by a glycosylated hemoglobin level <10% within 6 months of entrance into the study

b. Exclusion criteria

1. Concomitant nondiabetic renal disease, documented or suspected, or other known or suspected cause for microalbuminuria (collagen vascular disease, congestive heart failure, severe or uncontrolled hypertension, history of transplanted or single kidney)
2. Serum creatinine >1.5 mg/dl
3. Abnormal liver function, known active liver disease, or known carrier state for Hepatitis B or C
4. Concomitant use of supplemental Vitamin E or other antioxidant vitamins, including Vitamin A, Vitamin C, beta carotene, or probucol
5. Concomitant Warfarin therapy
6. A history of chronic nonsteroidal anti-inflammatory use, except daily single-dose aspirin
7. Pregnancy, or female subjects with plans for pregnancy within the next 12 months

G. Confidentiality of Study Data

All records pertaining to the subjects and data obtained in the study will be kept confidential; laboratory results will be included in each patient's confidential computerized medical record, while study materials, including the original signed consent form, will be kept in a secured filing cabinet in the office of the principal investigator.

H. Location of Study

All studies will be conducted at the Columbia Presbyterian Medical Center in the Renal Clinic offices, V-10, or in the Irving Center for Clinical Research.

I. Risks and Benefits

a. Risks

As noted above, all subjects will be maintained on standard therapy for diabetes throughout the study, under the care of their primary physician. No side effects of Vitamin E at this dose and duration in the absence of concomitant Warfarin therapy have been reported. Minimal risks my be expected related to biweekly phlebotomy, including hematoma formation or bleeding at the phlebotomy site.

b. Benefits

Although the goal of this study is to research a potential for benefit from Vitamin E therapy in patients with diabetic renovascular disease, no direct benefit to the patients in this short term study is to be expected.

J. Alternative Therapies

All subjects will continue standard therapy for diabetes and other conditions initiated prior to enrollment in the study.

K. Compensation and costs to subjects

No compensation for study participation will be offered.

L. Minors and research subjects

Not applicable

M. Radiation or radioactive substances

Not applicable.

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