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Long-term Alpha-Tocopherol Supplementation is Associated with Lower Serum Vascular Endothelial Growth Factor Levels

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Abstract. *Background: We previously reported that daily supplementation with a-tocopherol reduced prostate cancer risk in a large, randomized trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. One potential mechanism explaining this is that a-tocopherol inhibited tumor angiogenesis, an effect demonstrated in animal models. Patients and Methods: We evaluated whether long-term supplementation with α-tocopherol modified serum vascular endothelial growth factor (VEGF) levels, a cytokine integrally involved in angiogenesis, in men who were not diagnosed with cancer and had baseline and follow-up blood available. One hundred of these men who received α-tocopherol (50 mg daily) were randomly selected and matched on age, study center and time between blood draws to 100 men who received placebo (median follow-up 3.7 years). VEGF levels were measured by enzyme-linked immunosorbent assay. The effect of α-tocopherol supplementation on serum VEGF was evaluated using a matched-paired t-test for differences in the change in VEGF over the intervention period between groups. Results: There was an 11% reduction in VEGF levels in the α-tocopherol group as compared with a 10% increase in the placebo group (p=0.03). Conclusion: Our findings suggest that one of the mechanisms behind the inhibition of prostate carcinogenesis by α-tocopherol in the ATBC Study may have been through reduced VEGF concentrations and the suppression of tumor angiogenesis and therefore growth.*

Daily supplementation with α-tocopherol (vitamin E) resulted in a 32% reduction in the incidence of prostate cancer and a 41% reduction in mortality in the Alpha-Tocopherol, BetaCarotene Cancer Prevention (ATBC) Study (1). The mechanism by which α-tocopherol might have reduced prostate carcinogenesis is not known. However, since the reduction in incidence was observed within 2 years from the start of intervention and was greater for clinically overt tumors (stages II-IV) (1), it is suspected to have affected tumor growth or promotion.

Vascular endothelial growth factor (VEGF) is a cytokine that plays an important role in endothelial cell proliferation, vascular permeability and the regulation of physiological as well as pathological angiogenesis (2,3). VEGF is over-expressed in the majority of human cancers examined so far (4-7) and thought to promote tumor growth by the paracrine regulation of tumor angiogenesis. Blood VEGF levels have also been reported to be higher among cancer cases compared to controls and to bear prognostic significance (8-13). VEGF induction by oxidative stress has been demonstrated in experimental models (14, 15), and its induction *in vitro* was inhibited in the presence of antioxidants (15). Here, we evaluate whether long-term α-tocopherol supplementation altered serum VEGF concentrations in the ATBC Study.

Patients and Methods

Study participants. The current study consisted of a sample of 200 participants of the ATBC Study conducted in Finland who had not been diagnosed with cancer. The ATBC Study was a large, randomized, placebo-controlled prevention trial that tested the efficacy of 5-8 years of supplementation with α-tocopherol (50 mg daily as α-tocopherol acetate), ß-carotene (20 mg daily), or both in reducing the incidence of lung, prostate and other cancers. The trial cohort consisted of 29,133 male cigarette smokers between the ages of 50 and 69 who were recruited from southwestern Finland between 1985 and 1988 and followed during the active trial period until death or April 30, 1993. Men who had prior cancer, serious illness, or reported current use of vitamins A (> 20,000 IU/day), E (>20 mg/day), or ß-carotene (>6 mg/day) were ineligible. All study participants provided informed consent. Subjects for this substudy were randomly-selected from among trial participants who had both baseline and follow-up blood available and were on supplementation for at least two years. The time between the two blood draws ranged between 2.0 and 6.7 years (median 3.7 years). One hundred participants receiving only α-tocopherol supplementation were randomly-selected and matched to one hundred men receiving placebo on age, study center, and length of time between blood collections. Only men who consumed at least 90% of the study capsules were eligible.

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Table I. *Selected characteristics of study participants by intervention assignment ATBC Study, Finnish men.*

	Intervention group Medium (IR)*	
	Placebo (n=100)	α-tocopherol (n=100)
Characteristics		
Age (years)	54 (51-59)	54 (51-58)
Body mass index (kg/m^2)	25.4 (23.0-27.9)	26.0 (23.9-28.3)
Cigarettes (no. daily)	20 (15-25)	20 (15-25)
Alcohol (g/day)	9.1 (2.2-24.5)	10.3 (2.9-26.8)
Yrs between blood draws	3.7 (2.7-4.7)	3.7 (2.7-4.7)
Serum values		
Total cholesterol (mmol/L)*		
Baseline	6.2 (5.5-6.8)	6.4 (5.6-7.2)
Follow-up	5.9 (5.2-6.7)	6.1 (5.3-7.0)
α-tocopherol (mg/L)		
Baseline	11.2 (10.1-13.8)	11.8 (10.1-13.4)
Follow-up	12.5 (10.7-14.9)	17.9 (15.7-20.8)

*Median and interquartile distribution of VEGF

Table II. *Serum VEGF (pmol/L) of men receiving a-tocopherol supplementation from baseline to after 2 years of intervention, ATBC Study, Finnish men.*

	Serum VEGF Median (IR)*		p-value
	Placebo (n=100)	α-tocopherol (n=100)	
Baseline	249 (123-380)	251 (139-441)	
Follow-up	277 (128-399)	225 (126-412)	
Difference†	7.15 (-25.7-53.0)	-12.4 (-77.5-30.2)	
	10.4%	-11.2%	0.03ϋ

* Median and interquartile distribution of VEGF
† Median and interquartile range for the difference between follow-up and baseline serum VEGF levels according to group.
ϋ P-value based upon matched paired *t*-test.

Data collection. General medical history, diet and other background data along with a fasting blood sample were collected from all subjects at baseline. Participants made three follow-up visits annually and provided information on their health, use of non-trial vitamin supplements and smoking habits since their last visit. Follow-up blood from a random sample of 800 participants was taken annually beginning in the second year of the trial and onward. All subjects were asked to fast overnight and blood was collected in the morning. Serum was separated into 1.5 ml aliquots and stored at -70°C.

Baseline and follow-up serum samples for each matched pair were positioned adjacently within batches for analysis. Serum VEGF levels were measured by the Clinical Research Services (Frederick Cancer Research and Development Center, Frederick, MD, USA) using the ELISA kit following the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA). Each sample was ran in duplicate and the average value of the two used. Randomly placed blind quality control (QC) samples were placed within each batch. The overall coefficients of variations for QC serum VEGF was 34%.

Statistical analysis. All statistical analyses were performed using Statistical Analysis Systems software (SAS Corp, Cary, NC, USA). Differences in the study factors according to intervention assignment was assessed using the Wilcoxon rank sum tests. Any mean change in serum VEGF levels over the intervention period according to group was assessed using a matched paired *t*-test analysis. We also conducted multivariate linear regression to evaluate the association between change in VEGF levels with α-tocopherol supplementation in a matched analysis that adjusted for other study factors. Alcohol consumption, smoking, age, baseline α-tocopherol status, or BMI (at baseline or at time of follow-up serum collection) did not materially modify the associations (*i.e.*, by more than 10%). Effect modification was assessed by including factors and their cross-product terms with the treatment group variable in the multivariate regression models.

Results

The characteristics of the 200 participants according to intervention assignment are shown in Table I. There were no significant differences in baseline characteristics for age, weight, smoking, or alcohol consumption between the α-tocopherol or placebo groups, consistent with the randomized design of the original trial. There was a 50% increase in serum α-tocopherol in the supplement group after an average of nearly four years on supplements (range 2-7 years), compared to an 8% increase in the placebo group.

Serum VEGF concentrations (pmol/L) at baseline and at follow-up according to intervention group, along with the change in VEGF during the intervention period, are shown in Table II. Since VEGF concentrations were not normally distributed, the medians and interquartile ranges are presented. Serum VEGF levels were equivalent at baseline and decreased by 11.2% in the α-tocopherol group. In contrast, we observed a 10.4% increase in VEGF in the placebo group (p=0.03 for difference of the differences between groups).

We also evaluated potential interactions between intervention groups and time on intervention, age, years of smoking, number of cigarettes smoked α1 day and alcohol consumption. No material modification of the α-tocopherol effect was seen.

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Discussion

In an attempt to elucidate the mechanism(s) underlying the 32% reduction in prostate cancer incidence in the ATBC study, we measured serum VEGF in a subgroup of subjects either receiving α-tocopherol or placebo before and after nearly four years of supplementation and evaluated the differences between the changes in concentrations over time. We found a significant reduction in serum VEGF levels among subjects receiving daily supplementation of α-tocopherol compared to subjects receiving placebo. To our knowledge, this is the first time a relationship between α-tocopherol supplementation and serum VEGF has been demonstrated.

Our interest in VEGF was due, in part, to our trial findings suggesting that α-tocopherol affected tumor progression or promotion; *i.e.*, a greater risk reduction was observed for the clinically evident tumors (stage, II-IV), and a difference in risk between groups was observed within two years of intervention(1). Growth of solid tumors beyond a size of approximately 2-3 mm is dependent on the formation of new vessels(16) and VEGF has been demonstrated to play an integral role in this process (3).

Although a relationship between α-tocopherol and VEGF has not been directly demonstrated, other antioxidants have inhibited the induction of VEGF under conditions of oxidative stress. Acetylcysteine, a synthetic antioxidant, suppressed the induction of VEGF by oxidative stress (H_2O_2 application) in an endothelial cell line(14). A similar effect was observed in retinal epithelial cells, where antioxidants prevented the oxidative stress-induced VEGF increases (15). In experimental animals, α-tocopherol has been shown to inhibit angiogenesis, however, the mechanism has not been determined. Tumor size and angiogenesis in carcinogen-induced buccal pouch tumors were significantly inhibited in hamsters administered α-tocopherol compared to the placebo group (17).

There are several possible mechanisms explaining how α-tocopherol may have reduced serum VEGF levels. Induction of VEGF by oxidative stress (in endothelial cells) appeared to be mediated through protein kinase C (PKC) (14), which in turn, may be down-regulated by α-tocopherol (18). Thus, one plausible explanation is that α-tocopherol suppressed VEGF expression by inhibiting PKC activity, which is ubiquitously expressed. An alternative explanation is that serum VEGF levels were reduced as a result of the decrease in androgens produced by α-tocopherol supplementation: in a parallel nested study, we observed lower serum testosterone and androstenedione concentrations among subjects who received α-tocopherol (19). VEGF levels in the prostate have been shown to be regulated by androgens; castrated mice showed suppressed VEGF levels which were restored after exogenous administration of androgens (20,21).

A third explanation for our findings is that α-tocopherol inhibited VEGF release into the serum by inhibiting platelet aggregation, thereby creating an artifactual reduction in serum VEGF which does not reflect VEGF modulation at the tissue level. VEGF appears to be stored in alpha granules of platelets and to be released during their aggregation (22,23). Several studies have suggested that α-tocopherol inhibits platelet aggregation at higher concentrations (24), but the magnitude of this effect in our study is not known.

The results of the ATBC study suggest a prostate- (and possibly colon) specific effect of α-tocopherol supplementation. However, VEGF overexpression is implicated in the progression of many cancers. This apparent organ specificity in the ATBC study may be explained by the variability in tissue distribution of α-tocopherol. α-tocopherol is indeed a fat soluble vitamin transported into the cell along with cholesterol (25)and its concentration is known to be high in steroid-producing tissues, such as the prostate and the testes (26).

The possible effect of α-tocopherol on VEGF and angiogenesis may also have relevance for cardiovascular disease. VEGF has been implicated both in the response to myocardial ischernia. (27,28) as well as in atherosclerotic plaque formation (29). As far as the ATBC trial is concerned, no detrimental effect of α-tocopherol with regards to the incidence of major coronary events and fatal coronary disease was observed (30,31).

It is unclear whether serum, plasma or whole blood VEGF levels are most meaningful (32). Platelets contain VEGF, which is released consequent to platelet activation during coagulation (33) and some suggest that plasma (33,34) or whole blood levels (35) are therefore more appropriate. Our study was limited by a high coefficient of variation but, in spite of this, we were still able to detect statistically significant differences between groups in the VEGF assay.

In conclusion, serum VEGF concentrations were reduced among subjects receiving daily supplementation with α-tocopherol for an average of 3.7 years. This may have clinical implications for prostate cancer prevention since VEGF is a critical factor in tumor angiogenesis.

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