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Quantification of ï ¡-Tocopherol in Smokers and Non-Smokers Before and After Supplementation: Mechanism of Failure for Vitamin E in Prostate Cancer Prevention?

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Abstract

The primary endpoint of the study was to compare the levels of ï ¡-tocopherol and its oxidation products, ï ¡ï€-TQ and ï ¡ï€¬THQ, between smokers and non-smokers following 14 days of supplemental vitamin E. This study was a matched case control design that compared 20 smoking male subjects to 20 non-smoking male subjects, with subjects matched 1:1 with respect to age. Subjects were instructed to take one capsule of vitamin E 400 IU (all-rac-alpha-tocopheryl acetate) by mouth daily for 14 days with their morning meal. Samples were obtained on Day 1 (prior to vitamin E administration) and Day 15, approximately 24 hours after vitamin E administration for analysis of ï ¡-tocopherol and metabolites, NQO1 gene expression and cholesterol. The median baseline plasma ï ¡-tocopherol levels were significantly lower in smokers when compared to non-smokers, with values of 17.0 ug/mL (range 6.73-22.8) and 19.4 ug/mL (range 11.6-27.9) respectively, p=0.03. In both smokers and non-smokers, 14 days of supplementation with vitamin E significantly increased median ï ¡-tocopherol concentrations from baseline to a 23.3 ug/mL (range 10.7-37.5) and 29.8 (range 19.6-49.5) respectively, $p < 0.001$. ! After 14 days of vitamin E supplementation, non-smokers achieved plasma concentrations higher than the normal reference range, suggesting elevated plasma concentrations as a potential mechanism for increased prostate cancer risk in non-smokers receiving vitamin E.

Introduction

Prostate cancer will be diagnosed in approximately 180,890 men and account for 26,129 deaths in the United States in 2016, making prostate cancer the most commonly diagnosed and third leading cause of cancer death for American men [1]. Mortality related to prostate cancer has declined by more than 50% since 1990 [2]. In the last 25 years, annual PSA screening was both widely implemented and subsequently disproven as an effective prevention strategy and numerous chemoprevention clinical trials have failed to show a benefit; therefore, most experts attribute decreased prostate cancer mortality to improvements in treatment options [2]. While treatment options for prostate cancer are improving, treatments are both expensive and have adverse effects: urinary, sexual, and bowel-related adverse effects are common [3]. Given the prevalence, cost of treatment and associated adverse effects, primary prevention of prostate cancer remains an attractive option.

Preclinical and epidemiologic evidence supporting a role for selenium and vitamin E in prostate cancer risk reduction led to the implementation of a prospective randomized trial, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) [4] unfortunately demonstrating that vitamin E supplementation increased the risk of prostate cancer. The findings of SELECT are in contrast to those of the Alpha-Tocopherol, Beta Carotene (ATBC) trial [5], which reported a 35% risk reduction for prostate cancer in men taking 50mg daily of Vitamin E for a median of 6.1 years, as well as the Physicians Health Study [6] which showed no difference. Given that all the participants of ATBC were long term smokers compared to 43% never smokers and 8% current smokers in SELECT, this may suggest a differential benefit of vitamin E in smokers.

Vitamin E plasma concentrations are lower in smokers when compared to non-smokers potentially related to increased

oxidative stress [7-9] and subsequent increased need for the anti-oxidant vitamin E. Increased vitamin E needs may be a potential explanation for the prostate cancer chemoprevention effects of vitamin E supplementation in smokers, which appears to be absent in non-smokers [10]. Additionally, smokers may have altered vitamin E metabolism, where excessive oxidative stress results in increased generation of the vitamin E oxidation products ,α-tocopheryl quinone (,α –TQ) and ,α-tocopheryl hydroquinone (,α–THQ). Altered metabolism may be another potential mechanism for the differential chemoprevention effects of vitamin E. The primary endpoint of the study was to compare the levels of ,α-tocopherol and its oxidation products, ,α–TQ and ,α–THQ, between smokers and non-smokers following 14 days of supplemental vitamin E.

Materials and Methods

All aspects of the study protocol were reviewed and approved by the Institutional Review Board for human research at the University of Wisconsin in Madison, Wisconsin. This trial was completed before December 26, 2007 and was not required to be registered in ClinicalTrials.gov.

Patient Selection

Study participants were recruited at a single institution (University of Wisconsin, Madison) and were required to provide signed informed consent prior to enrollment. Patients were required to be men, without a history of cancer who were at least 40 years of age and were either current or former smokers. Current smokers were defined as having smoked more than 100 cigarettes in a lifetime and currently averaging at least one cigarette per day and non-smokers were defined as having smoked less than 100 cigarettes in a lifetime or smoked more than 100 cigarettes in a lifetime but quit more than 1 year ago. Patients were excluded if they were taking, or were unwilling to discontinue, vitamins, nutritional supplements, hormones, investigational agents or any medication interacting with Vitamin E or if they had an uncontrolled inter current illness.

Drug administration and study design

This study was a matched case control design that compared 20 smoking male subjects to 20 non-smoking male subjects, with subjects matched 1:1 with respect to age. Subjects were instructed to take one capsule of vitamin E 400 IU (all-rac-alpha-tocopheryl acetate) by mouth daily for 14 days with their morning meal. Study personnel performed a pill count at the day 15 visit. To be evaluable for analysis, 80% of the study drug (i.e., 11 capsules) must have been taken. Subjects who did not complete the study or took less than 80% of the study drug after 14 days were replaced.

Toxicity and Dose Modifications

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 3.0 [11]. The study drug was held for subjects developing grade 2 or greater toxicity considered possibly, probably, or definitely related to study drug.

Vitamin E and Metabolite Plasma, NQO1 mRNA and Serum Cholesterol Levels

Samples were obtained on Day 1 (prior to vitamin E administration) and Day 15, approximately 24 hours after vitamin E administration for analysis of ,α-tocopherol and metabolites, NQO1 gene expression and cholesterol. Plasma levels of ,α-–tocopherol [12], ,α–tocopheryl quinine [12] and ,α–tocopheryl hydroquinone [13] were assessed by the 3P laboratory at the University of Wisconsin Carbone Comprehensive Cancer Center (UWCCC) with a validated HPLC assay as previously described.

RNA was extracted from RNA Pax Gene tubes by standard methods and NQO1 was amplified by reverse transcription-polymerase chain reaction (RT-PCR). Gene expression levels were determined using iQ5 software (Bio-Rad, Hercules, CA). Expression was normalized using the housekeeping gene as a reference, and the first time point as the baseline. Serum cholesterol was evaluated in the UWHC clinical laboratory.

Statistical Considerations

This study was designed as a matched pair case-control study: 20 actively smoking male subjects and 20 non-smoking male subjects, with subjects matched 1:1 with respect to age. Based on the results from animal studies, it was anticipated that the plasma ,α-tocopherol levels would be higher among smokers than in non-smokers. Specifically, an effect size of 0.8 in plasma ,α–tocopherol levels for the difference between smokers and non-smokers was anticipated. The sample size of 20 smokers and 20 non-smokers provided 90% power for detecting an anticipated effect size of 0.8 at the two-sided 5% significance level.

Demographic information were presented in tabular format. Plasma ,α-tocopherol and normalized NQO1 expression levels were summarized in terms of medians and ranges. NQO1 expression levels were normalized to the day 1 values. The nonparametric Wilcoxon Signed Rank test was used evaluate changes in plasma tocopherol and relative NQO1 mRNA expression levels from day 1 to day 15 and to compare levels between matched paired smokers and non-smokers. The percentages of subjects with detectable α- tocopherol levels are summarized in frequency tables and an exact McNemar's test was used to evaluate changes from day 1 to day 15 and to compare smokers to non-smokers. Total cholesterol levels were summarized in terms of means and standard deviations and were analyzed using a paired t-test. All statistical tests were 2-sided, and a p-value of $< .05$ was used to indicate statistical significance. Statistical analyses were performed using SAS statistical software (SAS v9.222; SAS, Inc, Cary, NC).

Results

Baseline Subject Characteristics

This study enrolled 40 subjects, 20 smokers and 20 non-smokers. All subjects were male, had a performance status of 0, and the majority were White. Age ranged from 40-65 years

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and was similar between the groups. See (Tables 1 & 2). Vitamin E was well tolerated; all reported adverse effects were grade 1. Three subjects had constitutional symptoms, two subjects experienced pain, two had gastrointestinal disturbances and one had visual disturbances.

Table 1: Baseline Demographics.

	Smokers (n=20)	Non-Smokers (n=20)
Age (years)		
Median (range)	52 (40-65)	53 (41-64)
Gender		
Male	20 (100%)	20 (100%)
Race		
White	17 (85%)	19 (95%)
Black	2 (10%)	1 (5%)
Native American	1 (5%)	0 (0%)
ECOG Performance Status	0 (100%)	0 (100%)

Table 2: Plasma α-Tocopherol Concentrations.

	Smokers (n=20)	Non-Smokers (n=20)
Day 1	17.0 (6.73-22.8)	19.4 (11.6-27.9)*
Day 15	23.3 (10.7-37.5)	29.8 (19.6-49.5)*
Change Day 1- Day 15	6.3 (3.97-14.7)#	10.4 (8.00-21.6)#

*Significant difference between smokers and non-smokers, $p < 0.05$
#Significant difference between Day 1 and Day 15, $p < 0.001$

Vitamin E and Metabolite Plasma Levels

Plasma ,α-tocopherol and metabolite levels were measured at baseline and after 2 weeks of vitamin E administration (Day 15) in 20 male smokers and 20 matched controls as described in methods. The median baseline plasma ,α-tocopherol levels were significantly lower in smokers when compared to non-smokers, with values of 17.0 ug/mL (range 6.73-22.8) and 19.4 ug/mL (range 11.6-27.9) respectively, p=0.03. In both smokers and non-smokers, 14 days of supplementation with vitamin E significantly increased median ,α-tocopherol concentrations from baseline to a 23.3 ug/mL (range 10.7-37.5) and 29.8 (range 19.6-49.5) respectively, $p < 0.001$. While non-smokers had significantly higher plasma concentrations of ,α–tocopherol at baseline and after supplementation when compared to non-smokers, there was no significant difference in the change from baseline to Day 15 post-treatment between smokers and non-smokers, demonstrating that while smokers have lower endogenous ,α-tocopheral levels, supplementation with vitamin E is effective in increasing plasma levels. ,α-tocopherol metabolites were not detectable.

NQO1 mRNA levels in peripheral blood lymphocytes

Normalized NQO1 expression levels were used to analyze the changes from baseline to post-treatment assessment (Day 15), with the baseline values normalized to 1. Smokers had a median relative change in gene expression between Day 1 and 15 of 0.902 (range: 0.026-14.47), compared to non smokers with a median change in baseline between day 1 and 15 of 1.05 (range: 0.074-3.69). There were no significant differences between smokers and non-smokers identified in baseline or day 15 gene expression (data not shown) and no changes in expression after administration of vitamin E See (Figure 1), suggesting that NQO1 expression is not influenced by smoking status or vitamin E administration.

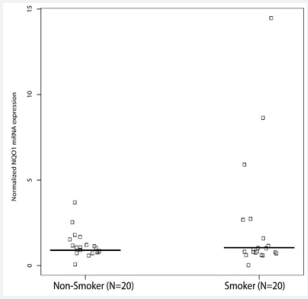

Figure 1: NQO1 gene expression was evaluated in peripheral blood mononuclear cells by semi-quantitative RT-PCR on day 1 and 15 in smokers and non-smokers. Normalized NQO1 expression levels were used to analyze the changes from baseline to post-treatment assessment (Day 15), with no significant differences identified between smokers and non-smokers. The horizontal bold lines represent the medians.

Discussion

The primary finding from this study is that plasma ,α-tocopherol levels are significantly lower in smokers than in non-smokers at baseline. This is consistent with prior literature [14] and supports the hypothesis that increased oxidative stress induced by smoking results in an increased demand for vitamin E. A novel finding of this work is that supplementation with 400IU of vitamin E is equally effective in increasing plasma ,α-tocopherol levels in both smokers and nonsmokers. Plasma concentrations in smokers increased a median of 6.3 ug/mL in smokers and 10.4 ug/mL in the non-smokers.

While baseline plasma levels of ,α-tocopherol were lower in smokers compared to non-smokers, both were within the normal references ranges of (5.5-20 ug/mL) [15] for adults at baseline. An additional finding of this study is that, after supplementation, non-smokers achieved a median plasma concentration of 29.8 ug/mL higher than the normal range with smokers also slightly outside the normal range at 23.3 ug/mL. Adverse effects in this 14 day study were mild and infrequent, however, a meta-analysis including 135,967 participants in 19 clinical trials evaluating vitamin E for cardiovascular or cancer benefits demonstrated that doses of vitamin E greater than or equal to 400IU/day were associated with a significantly increased risk of all cause mortality when compared to placebo controls. A dose-response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk

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associated with dosages greater than 150 IU/d, suggesting that commonly studied doses of vitamin E can have significant adverse effects and excess mortality [16].

The SELECT investigators [17] demonstrated that baseline ,α-tocopherol levels are not associated with an increased risk of prostate cancer. However, the intriguing question of whether elevated ,α-tocopherol concentrations after supra-therapeutic supplementation are a mechanism for increased risk of prostate cancer in this population remains unanswered. The dose used in most chemoprevention clinical trials was 400IU (nearly 20 times the recommended RDA) and it seems reasonable to anticipate, similar to this trial, that participants, particularly the non-smokers had elevated levels of ,α-tocopherol after years of therapy. Monitoring of plasma concentrations and supplementation only to the normal range should be considered in future trials of Vitamin E.

A limitation of the study is that subjects were not required to be fasting prior to plasma samples for ,α-tocopherol analysis being obtained, however we anticipate this potential measurement error is at least consistent between the groups. Additionally, the baseline ,α-tocopherol concentrations observed in this study are similar to those reported at baseline for the SELECT study participants, where quintile medians ranged from 8.64-20.09 ng/mL.

Conclusion

After 14 days of vitamin E supplementation, non-smokers achieved plasma concentrations higher than the normal reference range, suggesting elevated plasma concentrations as a potential mechanism for increased prostate cancer risk in non-smokers receiving vitamin E.

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