**Short Communication**

**Effect of β-Carotene Supplementation on Serum α-Tocopherol Concentration**

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Abstract

Conflicting reports of the effects of β-carotene supplementation on serum α-tocopherol concentration led us to evaluate serum α-tocopherol in subjects with and without β-carotene (30 mg/day) supplementation for up to 2 years duration in an ongoing chemoprevention trial. No adverse effect has been observed at any of the time periods examined.

Introduction

The efficacy of β-carotene as a chemopreventive agent is being tested in a number of Phase II and III trials (1). The potential of β-carotene is suggested by extensive epidemiological literature (2) and some animal experiments (3), but results from β-carotene chemoprevention trials to date are inconclusive with regard to efficacy (4–6). A 1992 report by Xu et al. (7) presented findings showing a decline in plasma and skin concentration of α-tocopherol after a 6-month supplementation with β-carotene. If total antioxidant capacity is a critical determinant of chemoprevention, then supplementation with one antioxidant that exerts antagonistic effects on other antioxidants will prove futile if not harmful. To better understand the results of micronutrient interventions, it is important to monitor for possible adverse interactions. We report here the results from an ongoing chemoprevention trial.

Materials and Methods

Study subjects ages 30–69 years were enrolled in a randomized, factorial design chemoprevention trial aimed at reducing the rate of progression of the gastric precancerous process. This trial was initiated in 1991 in Nariño, Colombia, an area with very high gastric cancer rates. A total of 863 men and women with histopathologically determined premalignant lesions of the stomach (multifocal chronic atrophic gastritis, intestinal metaplasia, or dysplasia) were randomly allocated to treatment with ascorbic acid (2 g/day) or placebo, β-carotene (30 mg/day) or placebo, and anti- Helicobacter pylori treatment (bismuth plus antibiotics) or no treatment. The trial is currently in progress.

Blood samples were obtained from all participants at baseline. Periodic random 10% samples are obtained to monitor compliance at the group level and to monitor serum concentrations of α-tocopherol and other nonsupplemented micronutrients. α-Tocopherol concentrations in subjects receiving β-carotene supplementation and placebo during the first 2 years are included in this analysis.

Blood was collected in vacutainers protected from light, centrifuged, and aliquots of serum were frozen at −70°C at the collection center in Colombia before shipment in dry ice to the laboratory located at Louisiana State University Medical Center (New Orleans, LA). Long-term storage (<2 years) before analysis was at −70°C.

The concentrations of β-carotene and α-tocopherol were determined by a modification of the method described by Craft et al. (8) by using reverse-phase HPLC with a C18 column. Each serum sample was thawed and centrifuged (3000 rpm; 5 min); serum (150 μl) plus 150 μl reagent alcohol (95:5:5 ethanol:methanol:isopropyl alcohol) were vortexed, 300 μl hexane were added, vortexed, and centrifuged. Two 100-μl hexane aliquots were evaporated under nitrogen in a 37°C water bath. One aliquot was dissolved in 100 μl 70:20:10 acetonitrile: dichloromethane:methanol (mobile phase) for carotenoid analysis, and the second aliquot was dissolved in 100 μl methanol (mobile phase) for the tocopherol analysis. The reference materials were obtained from the National Institute of Standards and Technology (Gaithersburg, MD). Two controls were analyzed with each sample run, one at the beginning of each sample series and one at the end. The sera were analyzed in batches. Approximately 20% of the pre- and postpaired samples were analyzed in the same batch.

To confirm that no α-tocopherol was present as an antioxidant preservative in the β-carotene capsules used in our trial, the contents were analyzed by HPLC. The capsules contained no detectable levels of α- or γ-tocopherol.

The differences in serum α-tocopherol before and after β-carotene or placebo supplementation were normally distributed. The mean differences in α-tocopherol concentration at follow-up and baseline by treatment group were evaluated by a paired t test. Each sampling period shown contains unique study subjects with no study subjects in more than one sampling period.

Results

Table 1 shows the mean serum concentrations of α-tocopherol by treatment arm for 5 periods of time on treatment: 1–3.5 months, 3.6–7 months, 7.1–12 months, 12.1–14 months, and >14 months (14–23). No decline in α-tocopherol concentration is observed at any time during the first 2 years of the study. A small but significant increase in α-tocopherol is observed after 7 months of supplementation; this increase is observed regardless of treatment assignment, placebo or β-carotene, after 12 months.

Within the β-carotene treatment arm, α-tocopherol con-
Table 1  Mean serum α-tocopherol concentration before and after β-carotene supplementation

<table>
<thead>
<tr>
<th>Time on treatment (months)</th>
<th>β-carotene treatment</th>
<th>n</th>
<th>Mean serum α-tocopherol concentration (µg/ml)</th>
<th>P value for treatment vs. placebo (Δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean (SEM)</td>
<td>P value Δ</td>
</tr>
<tr>
<td>1–3.5</td>
<td>Placebo</td>
<td>42</td>
<td>9.53 (0.37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>39</td>
<td>9.93 (0.44)</td>
<td>0.18 (0.45)</td>
</tr>
<tr>
<td>3.6–7.0</td>
<td>Placebo</td>
<td>39</td>
<td>8.09 (0.36)</td>
<td>0.56 (0.39)</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>42</td>
<td>9.11 (0.31)</td>
<td>0.45 (0.39)</td>
</tr>
<tr>
<td>7.1–12.0</td>
<td>Placebo</td>
<td>15</td>
<td>10.42 (0.72)</td>
<td>0.36 (0.60)</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>18</td>
<td>9.78 (0.75)</td>
<td>1.43 (0.57)</td>
</tr>
<tr>
<td>12.1–14.0</td>
<td>Placebo</td>
<td>36</td>
<td>9.22 (0.54)</td>
<td>1.65 (0.46)</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>38</td>
<td>10.21 (0.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;14.0</td>
<td>Placebo</td>
<td>23</td>
<td>9.49 (0.68)</td>
<td>1.00 (0.43)</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>28</td>
<td>9.05 (0.52)</td>
<td>1.09 (0.42)</td>
</tr>
</tbody>
</table>

Table 2  Studies of the effect of β-carotene supplementation on serum α-tocopherol concentration

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Population</th>
<th>β-carotene dose</th>
<th>Mean baseline α-tocopherol concentration</th>
<th>Duration of supplementation</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(7)</td>
<td>45 normal healthy nonsmoking males and females (U.S.)</td>
<td>15, 30, 45, 60 mg/day</td>
<td>7.88-8.44 µg/ml</td>
<td>9 months</td>
<td>40% decline from baseline after 9 months 15-60 mg/day β-carotene</td>
</tr>
<tr>
<td>(12)</td>
<td>14 normal male and female laboratory workers (U.S.)</td>
<td>30 mg/day</td>
<td>9.5 (0.6) µg/ml</td>
<td>16 weeks</td>
<td>No change</td>
</tr>
<tr>
<td>(13)</td>
<td>758 male asbestos workers (U.S.)</td>
<td>50 mg/day</td>
<td>13.2 µg/ml</td>
<td>2 yr</td>
<td>No change at 1 yr or 2 yr</td>
</tr>
<tr>
<td>(14)</td>
<td>222 male smokers (Finland)</td>
<td>20 mg/day</td>
<td>13.2 µg/ml</td>
<td>2 months</td>
<td>No change</td>
</tr>
<tr>
<td>(11)</td>
<td>241 male and female subjects with history of colon polyps (U.S.)</td>
<td>25 mg/day</td>
<td>12.6 ± 0.5 µg/ml and placebo 12.9 ± 0.4 β-carotene</td>
<td>1 yr</td>
<td>No change</td>
</tr>
<tr>
<td>(10)</td>
<td>34 subjects with history of colon polyps and 24 subjects with history of cancer (males and females; U.S.)</td>
<td>30 mg/day</td>
<td>Polyp patients: 1274.9 ± 462.7 µg/dl placebo, 1282.7 ± 276.8 µg/dl β-carotene</td>
<td>3 months</td>
<td>No change in polyp patients. Significant decline (P &lt; 0.01) in cancer pts on β-carotene and significant increase (P &lt; 0.01) in cancer pts on placebo</td>
</tr>
<tr>
<td>(9)</td>
<td>2319 male and female participants in CARET® pilot studies (asbestos exposed and smokers) and efficacy trial (U.S.)</td>
<td>15 mg/day (asbestos exposed); 30 mg/day (smokers and efficacy trial participants)</td>
<td>No baseline concentration for asbestos-exposed or smoker pilots. Efficacy study: (medians) 13.0 µg/ml β-carotene, 13.4 µg/ml placebo</td>
<td>6 yr</td>
<td>Significant (P = 0.03) increase in α-tocopherol after 2 yr</td>
</tr>
</tbody>
</table>

The “steady-state” serum concentration of α-tocopherol was similarly stable for males and females, as well as smokers and nonsmokers and persons treated for H. pylori infection compared to those not treated. The effect of supplementation with ascorbic acid on α-tocopherol concentration was also examined. No significant change in α-tocopherol concentration was observed at any time in participants taking ascorbic acid or placebo.

Discussion

No adverse effect of β-carotene supplementation on α-tocopherol serum concentrations was observed in our study, either short or long term. This finding adds to the growing number of large studies (see Table 2) that have failed to confirm the findings of Xu et al. (7).

Explanations for the small, but significant increase in α-tocopherol in the later sampling periods include laboratory drift and seasonal and/or temporal dietary changes. The data do not strongly support one of these possibilities over another. A blood sample taken from all active participants 36 months after entry into the trial will be available, and these data may clarify
this observation. At the present time, laboratory drift does not appear to account for the increase. An analysis of paired specimens run in the same batch for the later time periods exhibits significantly ($P = 0.005$) higher concentrations in the second sample compared to the first; however, this is based on only nine pairs. Nariño is a temperate region located in the Colombian Andes with only minor fluctuations in temperature year round. There are minimal seasonal dietary changes, with the exception of consumption of fresh fruits and vegetables, which reflects availability. Serum $\alpha$-tocopherol concentrations show no consistent monthly calendar effect. The shift toward higher levels appears temporal rather than seasonal. Multivitamins are not accessible in this population and so supplementation is not an explanation.

Goodman et al. (9) found no adverse effects after supplementation with $\beta$-carotene (30 mg/day) and vitamin A (25,000 IU/day) for up to 6 years in the largest study to date (8). Similar to our study, they also noted a statistically significant increase in serum $\alpha$-tocopherol, but the increase was not observed in participants taking placebo.

The only study to date other than Xu et al. (7) reporting a decline in $\alpha$-tocopherol concentrations in $\beta$-carotene supplemented subjects is a small randomized trial of 34 subjects with a history of colon polyps and 24 subjects with a history of colon cancer (10). After 3 months of supplementation with 30 mg/day $\beta$-carotene, no change in $\alpha$-tocopherol concentration was observed in subjects with a history of polyps, however, in the colon cancer series, a decline ($P < 0.01$) in serum $\alpha$-tocopherol was observed in the 12 patients taking $\beta$-carotene, whereas those patients on placebo showed an increase ($P < 0.01$). This study stands in contrast to much larger, longer term $\beta$-carotene intervention trials in which either no change, or a small but significant increase in $\alpha$-tocopherol, was found (8, 11–14).

This represents the first report from a developing country assessing the stability of serum $\alpha$-tocopherol concentration among participants in a $\beta$-carotene intervention trial. Our findings parallel those from trials in the United States and Finland: serum $\alpha$-tocopherol concentration appears stable in persons who take 30 mg/day of $\beta$-carotene for at least several years duration. This finding is consistent for both men and women, smokers and nonsmokers, with or without additional antioxidant supplementation, and without regard to the serum concentration of $\beta$-carotene achieved in persons taking supplements. It does not appear likely that the efficacy of $\beta$-carotene is measurably altered by an adverse interaction with serum $\alpha$-tocopherol concentration.

References


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