Effects of oral vitamin E and β-carotene supplementation on ultraviolet radiation–induced oxidative stress in human skin1–3

Frank McArdle, Lesley E Rhodes, Richard AG Parslew, Graeme L Close, Catherine IA Jack, Peter S Friedmann, and Malcolm J Jackson

ABSTRACT

Background: Ultraviolet radiation (UVR) generates reactive oxygen species in skin that can play a role in skin damage, but reports about the photoprotective properties of oral antioxidant supplements are conflicting.

Objective: We examined the ability of 2 lipid-soluble antioxidants, vitamin E and β-carotene, to reduce markers of oxidative stress and erythema in human skin exposed to UVR.

Design: Sixteen healthy subjects took either α-tocopherol (n = 8; 400 IU/d) or β-carotene (n = 8; 15 mg/d) for 8 wk. Biopsy samples before and after supplementation were taken from unexposed skin and skin 6 h after 120 mJ/cm2 UVR. The effects of supplements on markers of oxidative stress in skin and the minimal erythema dose to UVR were assessed.

Results: Supplementary vitamin E was bioavailable, the plasma concentration increased from 14.0 ± 0.66 (± SEM) to 18.2 ± 0.64 μg/mL (P < 0.01), and the skin concentration increased from 0.55 ± 0.09 to 1.6 ± 0.19 ng/mg protein (P < 0.01). Supplementary β-carotene increased plasma concentrations from 1 ± 0.3 to 2.25 ± 0.3 μg/mL (P < 0.05), but skin concentrations were undetectable. Before vitamin E supplementation, UVR increased the skin malondialdehyde concentration from 0.42 ± 0.07 to 1.24 ± 0.16 nmol/mg protein (P < 0.01), whereas oxidized or total glutathione increased from 9.98 ± 0.4% to 12.0 ± 1.0% (P < 0.05). Vitamin E supplementation significantly decreased the skin malondialdehyde concentration, but neither vitamin E nor β-carotene significantly influenced other measures of oxidation in basal or UVR-exposed skin.

Conclusions: Vitamin E or β-carotene supplementation had no effect on skin sensitivity to UVR. Although vitamin E supplements significantly reduced the skin malondialdehyde concentration, neither supplement affected other measures of UVR-induced oxidative stress in human skin, which suggested no photoprotection of supplementation.

KEY WORDS Lipid-soluble vitamins, oxidative stress, skin, photoprotection, human study

INTRODUCTION

Ultraviolet radiation (UVR) generates reactive oxygen species and free radicals in skin, which cause oxidation of cellular components, including lipid peroxidation in membranes (1). Considerable interest exists in the role of these substances in UVR-induced skin damage, including photocarcinogenesis, photosensitivity disorders, and photoaging (2). Several studies examined whether supplementary antioxidants can reduce the deleterious effects of UVR on skin, but no clear effects were established (2, 3). Our previous data indicated that exposure of skin fibroblasts in vitro (4) or human skin in vivo (5) to UVR leads to generation of reactive oxygen species with oxidation of biomolecules and induction of adaptive responses. Studies with fibroblasts showed that supplementation with Trolox, a vitamin E analog, suppressed UVR-induced oxidative stress in a reproducible manner, whereas other common dietary antioxidants did not (4).

Vitamin E is an important lipid-soluble antioxidant present within cellular membranes (1, 6), and interpretations of in vitro data indicate that carotenoids, such as β-carotene, can play similar roles as a lipid-soluble scavenger (7, 8). Both of these compounds previously were used in studies to reduce putative free radical damage in vivo with only limited success. Supplemental vitamin E protected against UVR-induced skin cancer in an animal model (9), whereas, in humans, vitamin E did not reduce clinical sunburn or numbers of histologically assessed sunburn cells (10). β-Carotene was partially successful in treatment of a photosensitivity disorder, erythropoietic protoporphyria, in which singlet oxygen is believed to be an important mediator (11). Several studies were performed to examine whether β-carotene protects against UVR-induced erythema in healthy humans, with widely differing reported effects (12–16). The incidence of nonmelanoma skin cancer was reported to be inversely related to serum β-carotene concentration (17), and earlier experimental UVR-carcinogenesis studies found β-carotene to be photoprotective (18, 19). However, the role of β-carotene as an anticancer agent was questioned as a result of randomized intervention studies in which supplementation did not reduce the incidence of nonmelanoma skin cancers in humans (20, 21). Some concern existed over the long-term use of high-dose β-carotene because findings indicate that supplements of this substance can have deleterious effects at higher doses in smokers (22).

1 From the Department of Medicine, University of Liverpool, Liverpool, United Kingdom.
2 Supported by the UK Ministry of Agriculture Fisheries and Food.
3 Reprints not available. Address correspondence to MJ Jackson, Department of Medicine, University of Liverpool, Liverpool, L69 3GA, United Kingdom. E-mail: m.j.jackson@liv.ac.uk.
Received December 19, 2003.
Accepted for publication April 26, 2004.

The aim of the work described here was to examine the effects of the 2 potential nutritional antioxidants, vitamin E and β-carotene, on tissue concentrations of these nutrients, markers of oxidative stress in normal human skin, before and after exposure to UVR and on UVR-induced erythema. Furthermore, β-carotene supplements were given at a low dose (15 mg/d), cognizant of the previous report of deleterious effects of higher doses (22). We hypothesized that supplementation of subjects with either vitamin E or β-carotene would reduce the oxidative stress in human skin induced by exposure to UVR in vivo.

SUBJECTS AND METHODS

Subjects and study design

Ethical permission for all studies was obtained from the Liverpool Research Ethics Committee, and investigations were conducted according to Declaration of Helsinki principles. Subjects gave written informed consent before participation. Sixteen healthy white volunteers (8 men, 8 women; age: 21 y; range: 18–27 y; skin type II or III) were recruited. None was exposed to MED and a 75% change in UVR-induced lipid peroxidation at this study were used in power calculations and indicated between-subject variability and likely magnitude of changes of UVR-induced lipid peroxidation by 200%. Data on the increased minimal erythema dose (MED) by 100% and a marker of lipid peroxidation in skin (23). This intervention supplementation on UVR-induced erythemal sensitivity and findings on the effect of a nutritional intervention with fish-oil (22). We hypothesized that supplementation of subjects which 10–12 mg was epidermal or dermal tissue. This tissue was separated from the rest of the biopsy sample, ie, fat, by dissection and split before homogenization in appropriate media for the biochemical analyses.

Skin samples

The skin biopsies provided 20 mg wet weight of tissue of which 10–12 mg was epidermal or dermal tissue. This tissue was separated from the rest of the biopsy sample, ie, fat, by dissection and split before homogenization in appropriate media for the biochemical analyses.

Biochemical analyses

Concentrations of vitamin E and β-carotene in plasma and skin were measured with the use of HPLC techniques modified to improve sensitivity by using electrochemical detection. β-Carotene and vitamin E were measured by the method of Catignani and Bieri (24) with electrochemical detection at an oxidation potential of +650 mV.

The malondialdehyde concentration of the sample was measured as an index of lipid peroxidation; the HPLC-based method of Fukunaga et al (25) was adapted to use small volumes of sample. The automated glutathione recycling method described by Anderson (26) was used to assess both the total and oxidized glutathione content of samples, with the use of a 96-well plate reader (Benchmark; Bio-Rad Laboratories, Hemel Hempstead, United Kingdom). Protein thiol concentration of samples was analyzed by the method of Di Monte et al (27), adapted for use on a 96-well plate reader. Catalase activity was measured by monitoring the enzymatic decomposition of hydrogen peroxide spectrophotometrically at 240 nm, with the use of the method of Claiborne (28). The protein content of samples was determined by using a bicinchoninic acid protein assay kit (Sigma Chemical Co, Dorset, United Kingdom), based on the method of Smith et al (29).

Skin erythema assessment

The MED was assessed before and after vitamin E and β-carotene supplementation. A geometric series (7–80 mJ/cm²) of UVR was applied in a horizontal row to buttock skin (Philips TL12 lamp), and the MED was defined as the lowest dose of UVR to result in a visually discernible erythema at 24 h.

Statistical analyses

Statistical analyses were carried out with the use of the Statistical Package for Social Sciences software, release 11.01 (SPSS Inc, Chicago). All data are presented as mean ± SEM. Simple comparisons of groups were undertaken with the use of Student’s paired t test. Where changes as a result of treatment and UVR exposure were compared, a 2-way analysis of variance with repeated measures was used to analyze all variables. When Mauchley test of sphericity indicated a minimal level of violation (0.75), the df was corrected with use of the Huynh-Feld adjustment. When sphericity was <0.75, the Greenhouse Geiser correction was used (30). When analysis of variance resulted in a significant F value, Tukey’s honestly significant difference test was used to explore where the differences occurred. Significance for all statistical tests was set at the α level < 0.05.

RESULTS

Effects of vitamin E supplements

Vitamin E supplementation increased both plasma vitamin E concentration (from 14.0 ± 0.66 μg/mL at baseline to 18.2 ± 0.64 μg/mL after supplementation; P < 0.01; Figure 1) and the concentration of vitamin E present in the skin (from 0.55 ± 0.09 to 1.6 ± 0.19 ng/mg protein; P < 0.01; Figure 2). Exposure of the skin to UVR did not consistently affect the vitamin E concentration.

A significant increase in malondialdehyde concentration was seen after UVR exposure both before (from 0.42 ± 0.07 to 1.24 ± 0.16 nmol/mg protein; P < 0.01) and after (from 0.24 ±
Vitamin E supplementation significantly decreased skin malondialdehyde concentration in the skin sample both exposed and not exposed to UVR. A significant decrease was observed in skin total glutathione concentration after UV exposure before supplementation which was not seen after vitamin E supplementation (Figure 3A). **Figure 4A**. UVR significantly increased the proportion of glutathione present in the oxidized form before supplementation, from 9.98 ± 0.4% in unexposed tissue to 12.0 ± 1.0% in UV-exposed skin (P < 0.05; Figure 4B). The proportion in the oxidized form also increased after UVR in the vitamin E–supplemented tissue. Supplemental vitamin E had no significant effect on the proportion of glutathione present in oxidized form.

A significant decrease was observed in skin total glutathione concentration after UV exposure before supplementation which was not seen after vitamin E supplements (Figure 4A). UVR significantly increased the proportion of glutathione present in the oxidized form before supplementation, from 9.98 ± 0.4% in unexposed tissue to 12.0 ± 1.0% in UV-exposed skin (P < 0.05; Figure 4B). The proportion in the oxidized form also increased after UVR in the vitamin E–supplemented tissue. Supplemental vitamin E had no significant effect on the proportion of glutathione present in oxidized form.

**Effect of vitamin E on the MED to UVR**

Vitamin E supplements had no effect on the visually observed erythemal response. Before and after supplementation, both median MEDs were 28 mJ/cm² (range: 20–28 mJ/cm²).
supplementation with vitamin E; significantly different from UVR exposed (\(P/L50141\) group. A significant supplementation was seen after UVR exposure both before and after vitamin E supplementation.

Effects of \(\beta\)-carotene supplements

\(\beta\)-Carotene supplementation increased the plasma \(\beta\)-carotene concentration from 1.0 \(\pm\) 0.3 \(\mu\)g/mL at baseline to 2.25 \(\mu\)g/mL \(\pm\) 0.3 \(\mu\)g/mL \(P < 0.05\); Figure 1) after supplementation. However, concentrations in skin were below the limit of detection for our HPLC method both before and after supplementation.

A significant increase in the skin malondialdehyde concentration was seen after UVR exposure both before and after \(\beta\)-carotene supplementation (Figure 3B). However, supplementation with \(\beta\)-carotene had no significant effect on either the basal or UVR-exposed skin malondialdehyde concentration.

Skin total glutathione was significantly increased after UVR exposure both before and after \(\beta\)-carotene supplements (Figure 5A). \(\beta\)-Carotene supplementation had no effect on the statistically significant increase in the proportion of the glutathione present in the oxidized form after UVR exposure (Figure 5B). The protein thiol concentration and catalase activity of skin were unaffected by either UVR exposure or \(\beta\)-carotene treatment (Table 1).

Effect of \(\beta\)-carotene on the MED to UVR

The elevation of plasma \(\beta\)-carotene concentration did not result in any significant effect on the MED. Before and after supplementation, both median MEDs were 28 \(mJ/cm^2\) (range: 20–36 \(mJ/cm^2\)).

DISCUSSION

Interpretation of our data indicates that oral supplementation with the lipid-soluble antioxidants, vitamin E or \(\beta\)-carotene, increased their respective plasma concentrations and that the skin vitamin E concentration also increased after this supplementation. Our finding of an increase in skin vitamin E concentration is in contrast to earlier work reporting no increase in skin concentrations after ingestion of a similar supplement of \(\alpha\)-tocopherol acetate for 1–6 mo (10). Skin \(\beta\)-carotene concentrations remained undetectable after supplementation. It is possible that an increase in skin \(\beta\)-carotene concentration could have occurred but with both control and supplemented concentrations remaining below the limit of analytic detection. However, the apparent lack of accumulation of \(\beta\)-carotene in skin concentrations is consistent with the absence of any effect of this low-dose supplementation on indicators of basal or UVR-induced oxidative stress. Garmyn et al (13) reported a significantly increased skin \(\beta\)-carotene concentration on analysis by HPLC, after supplements of 90 mg \(\beta\)-carotene/d for 3 wk, and Stahl et al (15) reported a significant increase in skin carotenoids, detected by noninvasive (spectrophotometric) techniques, after 25 mg total carotenoids/d for 12 wk or with 24 mg \(\beta\)-carotene/d for 12 wk (31). In further studies Stahl et al (32) could not demonstrate a change in skin carotenoids after 16 mg lycopene/d for 10 wk. A likely explanation for our lack of detection of an increase in skin concentrations is the lower supplements of \(\beta\)-carotene used in this study. We reasoned that previous reports of deleterious effects of \(\beta\)-carotene (22) caution against long-term use of high doses of this supplement and that studies of efficacy with lower doses of supplement were required. An alternative explanation for the lack of detection in our studies is that we eliminated subcutaneous fat from our analyses, and fat is known to have a higher \(\beta\)-carotene concentration in the epidermis and dermis (15). However, our findings are not consistent with any photoprotective effect with an additional 15 mg \(\beta\)-carotene/d.

Skin biopsies were obtained 6 h after UVR exposure because our previous findings indicated that at this time point a range of indicators of oxidative stress was apparent in skin biopsy samples (5). The irradiation source, which emits principally in the UVB (280–315 nm) wavelength, is capable of inducing mild oxidative stress in human skin (5). In the present study, we found significant elevations in malondialdehyde and oxidized glutathione (as a percentage of total glutathione) at 6 h after UVR, confirming the suitability of this protocol for assessment of these markers. Despite this confirmation, our studies revealed only a minor effect of vitamin E supplementation on the basal and UVR-induced increase in skin malondialdehyde, with no effect on the proportion of glutathione in the oxidized form. However, the vitamin E supplements did prevent the UVR-induced decrease in...
TABLE 1
Protein thiol content and catalase activity in skin samples before and after vitamin E and β-carotene supplementation

<table>
<thead>
<tr>
<th></th>
<th>Before vitamin E</th>
<th>After vitamin E</th>
<th>Before β-carotene</th>
<th>After β-carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>After UVR</td>
<td>Basal</td>
<td>After UVR</td>
</tr>
<tr>
<td>Protein thiol content (nmol/mg protein)</td>
<td>9.9 ± 0.7</td>
<td>10.1 ± 2.1</td>
<td>9.7 ± 1.4</td>
<td>10.0 ± 0.9</td>
</tr>
<tr>
<td>Catalase activity (U/mg protein)</td>
<td>124 ± 5</td>
<td>127 ± 7</td>
<td>119 ± 4</td>
<td>124 ± 3</td>
</tr>
</tbody>
</table>

All values are x ± SEM; n = 8 for each group. There were no significant effects of ultraviolet radiation (UVR) or of vitamin E or β-carotene supplementation on the protein thiol content or catalase activity in skin samples.

skin total glutathione content seen before supplementation. Consistent with these relatively minor biochemical changes, vitamin E did not protect against the visually assessed erythemal response to UVR. Studies in skin from hairless mice similarly showed no influence of dietary vitamin E concentration on UVR-induced malondialdehyde (33), whereas topical vitamin E was reported to reduce UVR-induced lipid peroxidation (33, 34). These differences can be attributable to the higher uptake of vitamin E observed after topical delivery (33). In a recent review, Anstey (35) concluded that in vitro studies showed potential for α-tocopherol as a systemic photoprotective agent, but in vivo studies so far failed to support this potential. Fuchs et al (36) similarly concluded that epidermal vitamin E concentration is not a determinant of individual photosensitivity.

We additionally studied the protein thiol concentration and catalase activity of skin biopsies after UVR exposure because our previous findings indicated that these measures were additionally influenced by UVR (5). The reason for the lack of changes in the current study (Table 1) is unclear.

Despite several studies indicating that β-carotene can act as a lipid-soluble antioxidant that reduces lipid peroxidation in membranes, no evidence for this was seen in the present work. One study reported that β-carotene supplements at doses of 30–90 mg/d produced a significant reduction in serum lipid peroxidation, although skin concentrations were not assessed (16). Our findings provide no support for low-dose β-carotene to induce a decrease in lipid peroxidation in skin. Several studies (15, 31, 32) supported a role for carotenoids in protecting against UVR-induced erythema, although the methods of assessment of UVR sensitivity differed in detail from those used here. The potential deleterious effects of higher doses of β-carotene in some population groups (22) urge caution in its widespread use as an oral photoprotective agent. Pro-oxidant effects of β-carotene were supported by findings from an animal model in which β-carotene augmented rather than protected against photocarcinogenesis (37). In that study, the researchers suggested that the previously observed protective effects of β-carotene in experimental UVR-induced carcinogenesis might be attributable to the conjoint action of other dietary factors as originally proposed by Black (38).

Experience with other putative antioxidants also was disappointing in this area. Supplementation with the water-soluble antioxidant, vitamin C, was found to induce a small reduction in basal concentrations of malondialdehyde in human skin, but it had no effect on UVR-induced skin oxidation (5). Moreover, evidence exists of down-regulation of endogenous skin antioxidants after vitamin C supplementation. It could be that combinations of oral antioxidants, or possibly higher-dose supplements, might be more efficacious in photoprotection. Ascorbate is known to regenerate tocopherol from the tocopherol radical formed during the antioxidant activity of vitamin E, hence promoting a synergistic action of the combined agents (39). This finding is supported by studies in which a significant difference in UVR-erythemal sensitivity was
reported on combined high-dose oral supplements of vitamins E and C (40, 41). Some similar findings also were reported for topical application of these 2 substances in animal models (42).

In summary, these findings show that supplementation of healthy subjects with vitamin E for 8 wk results in a significant increase in skin vitamin E concentrations, but no significant effects of vitamin E were seen on indicators of oxidative tissue damage in skin before or after UVR exposure. Similarly, low-dose β-carotene supplementation had no effect on basal or UVR-induced oxidation in skin. This work suggests that the use of single, low-dose, lipid-soluble antioxidants is an unpromising approach to systemic photoprotection.

We thank the late Tony Diplock for his helpful comments and Chris Price and Karen Brady for technical support. MJJ, LER, PSF, and CIAJ developed the original protocol for the study. FM and RAGP recruited the subjects and performed the ethyrmia and biochemical measurements. GLC performed the statistical analyses. All authors assisted in the interpretation and presentation of the results. The manuscript was drafted by FM with contributions from MJJ and LER. None of the authors had any conflict of interest that would have biased this work.

REFERENCES