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Ultraviolet B-Induced DNA Damage in Human Epidermis Is Modified by the Antioxidants Ascorbic Acid and D-α-Tocopherol

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DNA damage caused by ultraviolet (UV) irradiation is considered the main etiologic factor contributing to the development of skin cancer. Systemic or topical application of antioxidants has been suggested as a protective measure against UV-induced skin damage. We investigated the effect of long-term oral administration of a combination of the antioxidants ascorbic acid (vitamin C) and D-α-tocopherol (vitamin E) in human volunteers on UVB-induced epidermal damage. The intake of vitamins C and E for a period of 3 mo significantly reduced the sunburn reaction to UVB irradiation. Detection of thymine dimers in the skin using a specific antibody revealed a significant increase of this type of DNA damage following UVB exposure. After 3 mo of antioxidant administration, significantly less thymine dimers were induced by the UVB challenge, suggesting that antioxidant treatment protected against DNA damage.

Key words: human skin/ultraviolet radiation/antioxidants/DNA damage/thymine dimers
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Skin cancer is very frequent in Caucasians, and its incidence is increasing steadily. This development is caused inter alia by demographic changes (increased life expectancy) and by the intake of photosensitizing drugs (Placzek *et al*, 1999). These two effects together with an increased ultraviolet (UV) exposure by changes in the recreational behavior are a major cause of skin cancer. The exposure to UV radiation promotes the development of squamous cell carcinoma (SCC) and its precursor lesions, actinic keratoses. Epidemiologic data also imply UV as a major factor in the etiology of melanoma and basal cell carcinoma (BCC), although the role of the cumulative UV dose is less clear for these cutaneous malignancies than for SCC.

Both DNA damage caused by direct absorbance of UV radiation and indirect DNA damage contributed by reactive oxygen species (ROS) may lead to mutations, which can result in UV-induced skin cancer. The most prominent direct DNA damages are the cyclobutane pyrimidine dimers, i.e. thymine dimers, and the (6-4) photoproducts. Indirect DNA damage is inferred by ROS such as singlet oxygen ($^1O_2$) and free radicals such as the superoxide anion radical ($^{\bullet}O_2^-$), the perhydroxyl radical ($HO_2^{\bullet}$), or the hydroxyl radical ($^{\bullet}OH$).

Administration of antioxidants like ascorbic acid and D-α-tocopherol that scavenge ROS has been promoted as a strategy to decrease UV-induced skin damage and ultimately to also prevent skin cancer. In this study, we investigate the protective effect of long-term oral administration of antioxidant vitamins against UV-induced epidermal damage in human volunteers. We find that a combination of vitamins C and E decreased the sunburn reaction and protected epidermal cells against the induction of thymine dimers.

Abbreviation: UV, ultraviolet

Results

Subjects took 1 g of ascorbic acid and 500 IU of D-α-tocopherol twice daily over a period of 3 mo. None of the volunteers complained of any adverse events. The mean vitamin C serum level at the beginning was 11.6 ± 4.8 mg per liter (normal range: 4–20 mg per liter). After 1 mo of treatment vitamin C blood concentration was increased to 19.3 ± 7.5 mg per liter ($p < 0.001$). This increase remained stable, levels being 19.0 ± 6.8 mg per liter after 2 mo and 18.2 ± 6.8 mg per liter after 3 mo. Vitamin E serum levels were 21.0 ± 12.2 mg per liter at baseline (normal range: 5–20 mg per liter) and increased to 36.0 ± 14.4 mg per liter after 1 mo ($p < 0.001$). After 2 or 3 mo, they were 39.6 ± 17.9 or 38.8 ± 17.8 mg per liter, respectively. These data demonstrate that serum levels of vitamins E and C can be increased significantly by exogenous oral intake.

To assess the potential protective effects of antioxidant treatment against UVB-induced sunburn, we determined the minimal dose of UVB required to elicit a clearly demarkable erythema (reddening) of the skin on a previously unexposed body site (minimal erythema dose (MED)) before and after the 3 mo course of vitamins C plus E. After 90 d of antioxidant administration, the median MED rose from 80 to 113 mJ per cm^2 ($p = 0.002$, Wilcoxon's test; Fig 1) demonstrating that oral vitamins E plus C decreases the skin's susceptibility to sunburn.