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ULTRAVIOLET LIGHT-INDUCED GENERATION OF VITAMIN E RADICALS AND THEIR RECYCLING. A POSSIBLE PHOTOSENSITIZING EFFECT OF VITAMIN E IN SKIN

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Vitamin E (α-tocopherol) is the major lipid-soluble chain-breaking antioxidant of membranes. Its UV-absorbance spectrum (λ_{max} 295 nm) extends well into the solar spectrum. We hypothesize that in skin α-tocopherol may absorb solar UV light and generate tocopheroxyl radicals. Reduction of tocopheroxyl radicals by other antioxidants (e.g. ascorbate, thiols) will regenerate (recycle) vitamin E at the expense of their own depletion. Hence, vitamin E in skin may act in two conflicting manners upon solar illumination: in addition to its antioxidant function as a peroxyl radical scavenger, it may act as an endogenous photosensitizer, enhancing light-induced oxidative damage. To test this hypothesis, we have illuminated various systems (methanol-buffer dispersions, liposomes and skin homogenates) containing α-tocopherol or its homologue with a shorter 6-carbon side chain, chromanol-alpha-C6 with UV light closely matching solar UV light, in the presence or absence of endogenous or exogenous reductants. We found that: (*i*) α-tocopheroxyl (chromanoxyl) radicals are directly generated by solar UV light in model systems (methanol-water dispersions, liposomes) and in skin homogenates; (*ii*) reducing antioxidants (ascorbate, ascorbate + dihydrolipoic acid) can donate electrons to α-tocopheroxyl (chromanoxyl) radicals providing for vitamin E (chromanol-alpha-C6) recycling; (*iii*) recycling of UV-induced α-tocopheroxyl radicals depletes endogenous antioxidant pools (accelerates ascorbate oxidation); (*iv*) β-carotene, a non-reducing antioxidant, is not active in α-tocopherol recycling, and its UV-dependent depletion is unaffected by vitamin E.

KEY WORDS: Vitamin E, α-tocopherol, free radicals, recycling, skin, liposomes, ascorbate, dihydrolipoic acid, β-carotene, UV

INTRODUCTION

Solar ultraviolet (UV) radiation is implicated in the genesis of many types of skin damage (erythema, photodermatoses, photoaging, skin fragility, skin cancer etc.), the most harmful of which are melanoma and non-melanoma skin cancer.[1–4] UVB light (290–320 nm) is considered to be the portion of the UV spectrum primarily responsible for these deleterious effects of solar UV light.[2,5] However, the endogenous photosensitizer(s) triggering UVB-induced damage in the skin has not been identified.[6–8] Much evidence indicates that photodamaging processes, especially cancer, may be mediated by free radicals: free radicals have been detected in UV-illuminated lyophilized epidermis[9] and in UV-illuminated human skin,[10,11] chemically-induced

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skin cancer appears to be mediated by free radicals[12-14] and antioxidant enhancement reduces carcinogenesis in skin, both for chemically-induced carcinogenesis[15-18] and for UV light-induced carcinogenesis.[19-21] In particular, lipid peroxyl radicals have been linked to carcinogenesis in skin induced by chemical treatment[22] and by UV light,[21] and an accumulation of lipid peroxidation products has been found in UV-illuminated skin.[21,23]

The major chain-breaking antioxidant in membranes is vitamin E,[24,25] and vitamin E is present in the skin.[26-28] The vitamin E absorbance spectrum (maximum at 295 nm) extends well into the solar spectrum.[29] This raises the possibility that vitamin E itself may absorb solar UV light and become a free radical (the tocopheroxyl radical). It is known that reductive antioxidants (ascorbate, thiols, ubiquinols, etc.) can reduce tocopheroxyl radicals back to tocopherol molecules (recycling of tocopherol);[30-34] in this process intermediate radicals and final oxidized forms of these antioxidants are produced and the antioxidants are depleted. It thus appears that vitamin E in skin may absorb UV light, generating the tocopheroxyl radical, and, by the mechanism of its own recycling, deplete other antioxidants. Hence, vitamin E in skin may act in two conflicting manners — as a peroxyl radical scavenger, and as an endogenous photosensitizer enhancing light-induced oxidative damage.

To test this hypothesis, we have illuminated various systems containing vitamin E (methanol–water dispersions, liposomes and skin homogenates) with UV light closely matching solar UV light, in the presence or absence of endogenous or exogenous reductants.

MATERIALS AND METHODS

Samples

Methanol-buffer suspension. d-α-Tocopherol was initially dissolved in pure methanol and dispersed to a concentration of 3.0 mM in methanol : phosphate buffer mixture (4 : 1, v/v, phosphate was 50 mM, pH 7.4) for ESR measurements. The methanol was HPLC-grade (Fisher), and showed no absorbance peaks in the wavelengths emitted by the solar simulator.

Liposomes (α-Tocopherol + Ascorbate)-system. d-α-Tocopherol in ethanolic solution was added to dioleoylphosphatidylcholine (DOPC) in chloroform. The combined solution was evaporated to dryness under a stream of nitrogen; phosphate buffer (50 mM, pH 7.4 at 25°C) was added and multilamellar liposomes were obtained by vigorous shaking. The final concentration of DOPC in liposomal suspension was 20 mM and of α-tocopherol was 1.8 mM for the ESR measurements. In experiments in which ascorbate consumption was measured the DOPC concentration was 0.5 mM. Based on the measurements of α-tocopherol fluorescence intensity in DOPC liposomes in the presence and in the absence of a detergent (sodium deoxycholate, 25 mM), this procedure provides for a complete incorporation of α-tocopherol into liposomes.[35]

(α-Tocopherol + β-carotene)-system. d-α-Tocopherol in ethanolic solution and β-carotene in hexane solution were added to DOPC in chloroform. The combined solution was evaporated to dryness under a stream of nitrogen; phosphate buffer (50 mM, pH 7.4 at 25°C) was added and the liposomal suspension was obtained as above. The final concentration of both α-tocopherol and β-carotene in the suspensions was 50 μM and that of DOPC was 0.5 mM.

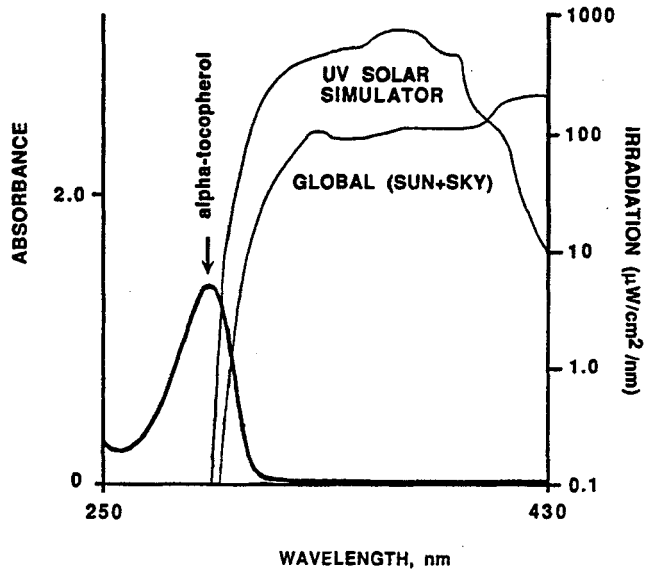

FIGURE 1 Comparison of UV-absorbance spectrum of an ethanolic solution of α-tocopherol with the spectrum of global (sun emission + sky) overhead sun light and of the light emitted by the solar UV-simulator. α-Tocopherol concentration was 0.4 mM.

Skin homogenates. Hairless mice (Simonsen, strain SKH-1) were used as a skin source. Skin samples were removed immediately after sacrifice of the animal by CO_2 asphyxiation. Skin homogenates were prepared as described previously.[26] For E-enriched skin, d-α-tocopherol was applied topically in excess, for one hour. The mouse was then killed, the skin washed 10 times with detergent (Joy dishwashing liquid) to remove excess E that did not penetrate the skin, and the homogenate prepared as above. Skin homogenates enriched with α-tocopherol or its homologue with the 6-carbon side-chain (chromanol-alpha-C6) were obtained by the addition of ethanolic solutions of α-tocopherol (chromanol-alpha-C6) to the homogenates. The suspensions were vigorously shaken, and incubated for 10 min, after which ESR spectra were recorded.

Irradiation. UV light was provided by a simulated solar light source (model 14S, Solar Light Co, Phila., PA) which covers the wavelength range from 295 to 400 nm in a pattern similar to natural sunlight (Figure 1). The power density of the light at the sample surface in the spectral region 310–400 nm was 1.5 mW/cm^2 and dropped to 10% of this value at 295 nm. The samples were illuminated in the ESR resonator cavity (in the course of ESR measurements) or in a quartz cuvette (measurements of tocopherol, ascorbate or β-carotene consumption); the distance between the light source and the sample was 30 cm. Oxygen was bubbled through the solution prior to and during irradiation.

ESR measurements. ESR measurements were made on a Varian E 109E spectrometer at room temperature, in gas permeable Teflon tubing (0.8 mm internal diameter, 0.013 mm thickness obtained from Zeus Industrial Products, Raritan N.J. USA). The

gas permeable tube (approximately 8 cm in length) was filled with 60 μl of a mixed sample, folded into quarters and placed in an open 3.0 mm internal diameter EPR quartz tube such that all of the sample was within the effective microwave irradiation area.

ESR spectra were recorded either in the dark or under continuous UVAB irradiation by the solar simulator in the ESR cavity. Spectra were recorded at 100 mW power and 2.5 Gauss modulation, and 25 Gauss/min scan time for liposomes and skin homogenates. For methanol–water suspensions ESR setting were the same, except the power was 10 mW, and the modulation amplitude was 1.6 Gauss. Spectra were registered under aerobic conditions by flowing oxygen through the ESR cavity, at 3245 Gauss magnetic field strength and scan range 100 Gauss, time constant 0.064 s.

HPLC measurements of α-tocopherol content. α-Tocopherol was assayed by reverse phase HPLC using a C-18 column (Waters, Inc.) with an in-line electrochemical detector. The eluent was methanol : ethanol 1 : 9 (v/v), 20 mM lithium perchlorate. Tocopherol was extracted into hexane from sodium dodecyl sulfate-treated samples as described earlier.[36]

HPLC measurements of β-carotene content. β-Carotene concentrations were determined by HPLC using a Beckman/Altex system with an Ultrasphere ODS, 5 mm, 4.6 × 150 mm reverse phase column (Beckman) and elution solvent composition of 82.5% methanol, 17.5% toluene, and 0.5% ammonium acetate at a flow rate of 1 ml/min. The effluent was monitored at 450 nm by a spectrophotometric detector for β-carotene. To extract 200 μl samples, an equal volume of ethanol plus 0.025% butylated hydroxytoluene (BHT) was added. After saturating with sodium chloride and adding 5 μl of a solution of lycopene in tetrahydrofuran (as an internal standard), the samples were extracted twice with 6 volumes of hexane plus 0.025% BHT. The hexane was evaporated to dryness under nitrogen and reconstituted in 230 μl of a mixture of toluene/methanol (1 : 3) for HPLC analysis.

Determination of ascorbate concentration. The changes in the ascorbate concentration (the initial concentration was 140 μM) in the presence of liposomes containing various amounts of α-tocopherol was monitored spectrophotometrically (λ_{max} 265 nm, $\varepsilon = 14\,500\ M^{-1}cm^{-1}$).[37] Serial spectra were recorded at 10 min intervals, in the spectral region from 220 to 360 nm, during the course of irradiation of the solution by the solar simulator. The highest α-tocopherol concentration in these measurements was 100 μM. α-Tocopherol with its low molar absorbance in the maximum ($\varepsilon_{295} = 3265\ M^{-1}cm^{-1}$)[29] did not interfere with the photometric assay of ascorbate at 265 nm.

Reagents. DOPC, β-carotene, and ascorbate were from Sigma Chemical Company, St. Louis, MO; potassium phosphate dibasic, sodium phosphate monobasic were from Mallinckrodt, Inc. Paris, KY; HPLC grade ethanol and methanol were from Fischer Scientific, Fair Lawn, N.J. 2R,4R′,8R′-α-tocopherol was a gift from Henkel Co. Dihydrolipoic acid and lipoic acid were a gift from Asta Pharma. Chromanol-alpha-C6 was synthesized and kindly provided by Prof. R.P. Evstigneeva (Institute of Fine Chemical Technology, Moscow, USSR).

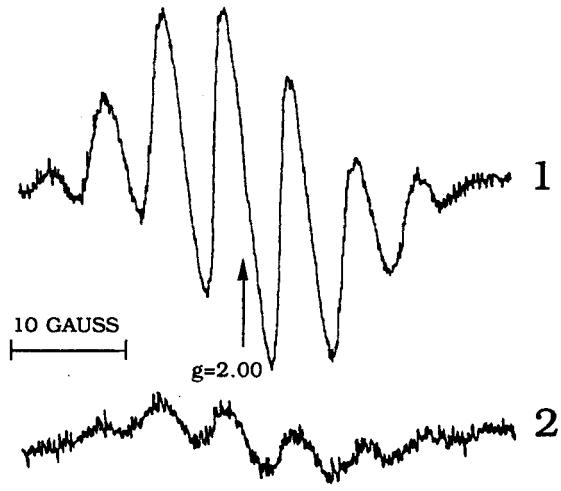

FIGURE 2 ESR spectra of tocopheroxyl radicals in methanol–water dispersions and DOPC liposomes. (1) UV-induced tocopheroxyl radical signal from vitamin E (3.0 mM) dispersed in a methanol-buffer mixture. (2) UV-induced tocopheroxyl radical signal in vitamin E-containing DOPC liposomes; 2 min of illumination, the d-α-tocopherol concentration was 1.8 mM.

RESULTS AND DISCUSSION

Generation of Vitamin E Radicals in Methanol–Water Suspensions and in Liposomes

When methanol–water suspensions or DOPC liposomes containing α-tocopherol were illuminated with simulated solar UV light, the tocopheroxyl radical electron spin resonance (ESR) signal appeared immediately (Figure 2); in the dark the tocopheroxyl radical signal was not present.

We used a set of cut-off spectral filters to evaluate the spectral sensitivity of UV-induced generation of tocopheroxyl radicals from α-tocopherol (absorbance maximum at 295 nm) in DOPC liposomes. We found that the filter absorbing UV-light in the range 264 nm and shorter (at 264 nm absorbance was 2.0) did not affect the tocopheroxyl radical signal. The filter absorbing UV up to 315 nm (at 315 nm absorbance was 2.0) completely prevented the appearance of tocopheroxyl signal. The two filters cutting off the UV-light in between these values caused partial quenching of tocopheroxyl radical ESR signal: the filter absorbing up to 282 nm (at 282 nm the absorbance was 2.0) decreased the magnitude of the signal only by 17%, while the filter absorbing UV up to 305 nm (at 305 nm the absorbance was 2.0) quenched the signal by 71%. Thus, partially or completely filtering the light in the wavelengths of the absorption spectrum of vitamin E during illumination of liposomes partially or completely eliminated the tocopheroxyl radical ESR signal.

The overlap of the absorbance spectrum of α-tocopherol with the solar spectrum (Figure 1), the absence of other photosensitizers in the methanol and liposomal preparations, and the elimination of the tocopheroxyl radical ESR signal in illuminated systems when light in the absorbance region of α-tocopherol was removed indicate that the generation of tocopheroxyl radicals was due to the direct effect of UV light on α-tocopherol and was not mediated by other photosensitizers.

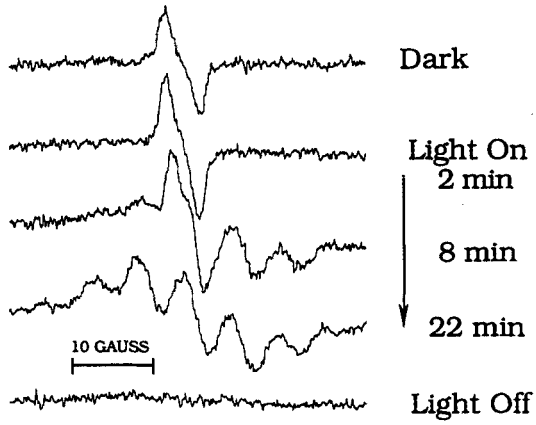

FIGURE 3 Ascorbyl and tocopheroxyl ESR radical signals in preparation of (DOPC + α-tocopherol) liposomes suspended in ascorbate solution (4.2 mM ascorbate, final α-tocopherol concentration in suspension 1.8 mM). For incubation and ESR recording conditions see Methods.

Recycling of Vitamin E in Liposomes and Antioxidant Depletion

To test whether the tocopheroxyl radicals induced by UV light could deplete other antioxidants which serve as intracellular reductants, we illuminated DOPC liposomes containing α-tocopherol in the presence or absence of ascorbate. For (DOPC + α-tocopherol) liposomes suspended in buffer without ascorbate, the appearance of the tocopheroxyl ESR signal was again immediate upon illumination, while for ascorbate solutions in which liposomes containing DOPC only were suspended, the ascorbyl radical ESR signal appeared in the dark (Figures 3, 4), and was not affected by UV illumination, staying relatively constant over time (Figure 4A). This dark signal was probably due to aerobic oxidation of ascorbate. In preparations of (DOPC + α-tocopherol) liposomes suspended in ascorbate solution, upon illumination there was an immediate increase in the ascorbyl radical ESR signal (Figure 3), which then decayed rapidly, while there was a delay in the appearance of the tocopheroxyl radical ESR signal indicative of the recycling of the tocopheroxyl radicals by ascorbate (Figure 4B). Only after the ascorbyl radical signal dropped below its initial dark value (i.e., the value shown in Figure 4A) did the tocopheroxyl radical ESR signal appear; the tocopheroxyl radical signal did not reach the intensity seen in systems lacking ascorbate until the ascorbyl radical ESR signal had disappeared completely, at about 16 min of illumination.

In another experiment we added to the system dihydrolipoic acid (DHLA), a reductant with a more negative redox potential (−0.32 V for the couple lipoate/dihydrolipoate) than ascorbate (0.08 V for the couple dehydroascorbate/ascorbate), which is thus able to regenerate ascorbate from its oxidation products.[38] DHLA enhanced the effect of ascorbate on α-tocopherol recycling in DOPC liposomes: the tocopheroxyl radical ESR signal could not be detected even after 24 min of UV illumination, and the ascorbyl radical ESR signal remained at or above baseline intensity for the same period (Figure 4C). In the absence of ascorbate, DHLA only slightly decreased the magnitude of the tocopheroxyl radical ESR signal without significant change of its time-course, i.e. it did not directly reduce tocopheroxyl

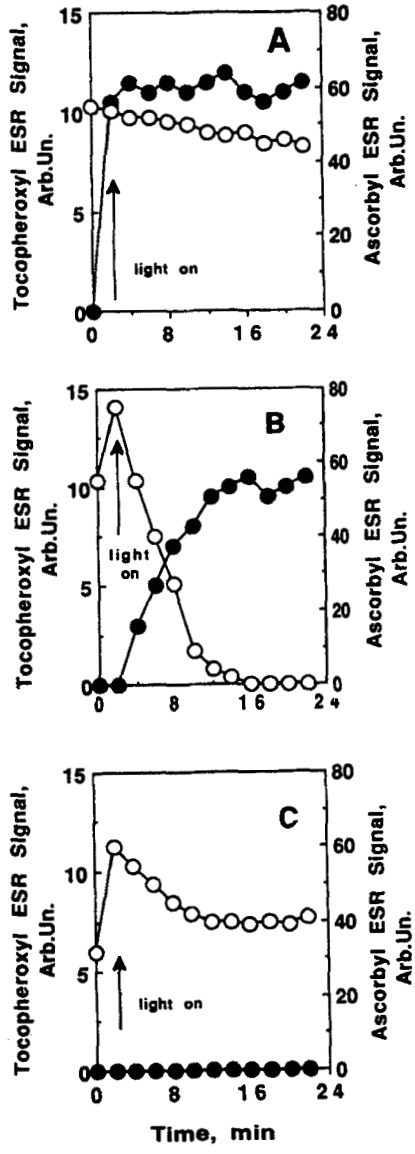

FIGURE 4 Time course of UV-induced ESR signals of tocopheroxyl and ascorbyl radicals in DOPC liposomes suspended in phosphate buffer (50 mM, pH 7.4 at 25°C). For incubation and ESR recording conditions see Methods; (- - -○- - -) ascorbyl radical signal; (- - -●- - -) tocopheroxyl radical signal. (A) Ascorbyl radical signal in illuminated preparations of DOPC liposomes (no α-tocopherol) suspended in 4.2 mM ascorbate; tocopheroxyl radical signal in illuminated preparations of (DOPC + tocopherol) liposomes liposomes in buffer (no ascorbate, final tocopherol concentration in suspension 1.8 mM); (B) Ascorbyl and tocopheroxyl ESR radical signals in illuminated preparation of (DOPC + α-tocopherol) liposomes suspended in ascorbate solution (4.2 mM ascorbate, final α-tocopherol concentration in suspension 1.8 mM); (C) Same as (B), plus dihydrolipoic acid (4.0 mM).

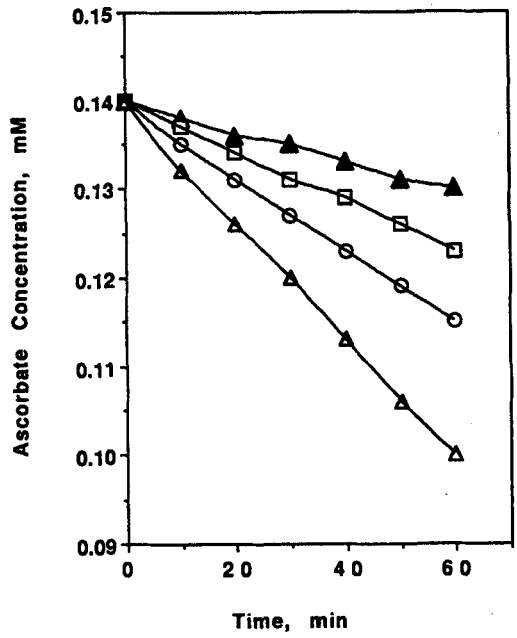

FIGURE 5 Time course of UV-induced ascorbate consumption in DOPC liposomes in the presence of α-tocopherol. Liposomes were prepared as described in Methods. Final α-tocopherol concentrations in the suspensions were 0 (control) (---▲---), 25 (---□---), 50 (---○---) and 100 μM (---△---) (1 : 20, 1 : 10 and 1 : 5 α-tocopherol : DOPC ratios). Ascorbate was added to liposomal suspensions to give a final concentration 0.14 mM. Samples were illuminated by the solar simulator in a manner similar to that used for ESR measurements, except the samples were in a quartz cuvette. Oxygen was bubbled through the solution prior to and during illumination.

radicals. Lipoic acid, the oxidized form of DHLA, was not efficient in vitamin E recycling, either in the presence or in the absence of ascorbate.

The results are consistent with the preservation of the tocopherol pool in the illuminated liposomes through recycling of the UV-light-induced tocopheroxyl radical back to tocopherol at the expense of reducing antioxidants. We therefore measured the consumption of ascorbate in illuminated suspensions containing DOPC liposomes with various amounts of α-tocopherol added, and the sparing of α-tocopherol by reductants. These measurements showed that under illumination by UV-light α-tocopherol stimulates oxidation of ascorbate in a concentration-dependent manner (Figure 5). Conversely, in illuminated preparations of (DOPC + α-tocopherol) liposomes suspended in solutions containing ascorbate alone, DHLA alone, or (ascorbate + DHLA), ascorbate had a partial α-tocopherol-sparing effect, DHLA alone had almost no α-tocopherol-sparing effect, and (ascorbate + DHLA) almost completely preserved α-tocopherol against UV-induced consumption (Table I).

When α-tocopherol-containing DOPC liposomes were loaded with β-carotene, another lipid-soluble antioxidant which does not possess reducing potency, the UV-induced α-tocopheroxyl radical ESR signal was unaffected, indicating that no recycling of α-tocopherol occurred. β-Carotene also did not have an α-tocopherol-sparing effect under UV illumination (Table II). The presence or absence of

TABLE I
UVB-Induced consumption of α-tocopherol in DOPC liposomes

Conditions	α-Tocopherol, μM
Control (before UV-irradiation)	50.0
+ UV-irradiation (60 min)	16.7 ± 2.4
+ UV-irradiation + ascorbate	31.4 ± 4.0
+ UV-irradiation + dihydrolipoic acid	22.9 ± 2.2
+ UV-irradiation + ascorbate + dihydrolipoic acid	47.8 ± 4.2

α-Tocopherol (50 μM) was incorporated into DOPC liposomes; the concentration of DOPC in phosphate buffer (50 mM, pH 7.4 at 25°C) was 320 μM. The mean values ± S.E. (n = 3) are given.

α-tocopherol in DOPC liposomes had no effect on the decrease in β-carotene concentration under UV illumination (Table II).

These results indicate that UV illumination of α-tocopherol in DOPC liposomes depletes ascorbate, and that, conversely, these antioxidants spare α-tocopherol from destruction. However this interplay is only possible between α-tocopherol and reducing antioxidants which are capable of donating reducing equivalents to tocopheroxyl radicals.

Generation of Vitamin E Radicals and Their Reduction in Skin Homogenates

To investigate these phenomena in a more physiological system, we used homogenates of hairless mouse skin. In freshly prepared non-illuminated skin homogenates, an endogenous ascorbyl radical ESR signal was observed (Figure 6) in agreement with previous reports.[37] The identity of the ascorbyl free radical signal was confirmed by comparing the intrinsic signal with that obtained after addition of: (*i*) pure sodium ascorbate to the skin homogenate (the magnitude of the signal increased) or (*ii*) ascorbic acid oxidase (the signal transiently increased, then disappeared). Without illumination the intensity of the signal decreased with time, and after 30 min the signal could hardly be detected (data not shown). When the sample of the normal skin homogenate was illuminated by the solar simulator, the intensity of the ascorbyl free radical signal increased by 25–30%, and the decay of the signal was more rapid than in the dark. No ESR tocopheroxyl radical signal from endogenous vitamin E was observed in normal skin homogenates (3.2 nmol of α-tocopherol/g tissue) after disappearance of ascorbyl radical signal. Tocopheroxyl radical ESR signal appeared faintly after ascorbate depletion in homogenates from skin to which α-tocopherol had been topically applied (104 nmol of α-tocopherol/g tissue).

TABLE II
UVB-induced consumption of α-tocopherol and β-carotene in DOPC liposomes

	α-Tocopherol, μM		β-Carotene, μM	
Time (min)	− β-carotene	+ β-carotene	− α-tocopherol	+ α-tocopherol
Control	50.0	50.0	50.0	50.0
30	30.2 ± 3.5	30.1 ± 2.9	3.0 ± 0.2	2.9 ± 0.2
60	16.7 ± 2.4	15.8 ± 2.0	0.9 ± 0.1	0.9 ± 0.1

Both α-tocopherol and β-carotene were incorporated into DOPC liposomes; the concentration of DOPC in phosphate buffer (50 mM, pH 7.4 at 25°C) was 320 μM. The mean values ± S.E. (n = 3) are given.

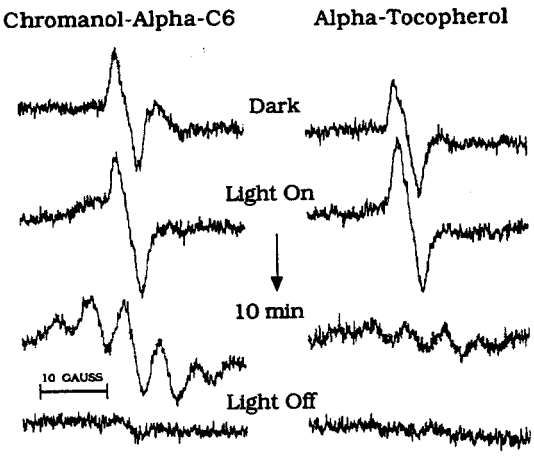

FIGURE 6 Ascorbyl, α-tocopheroxyl and chromanoxyl ESR radical signals in homogenates from skin of hairless SKH-1 mice. α-Tocopherol (0.780 μmol/g of tissue) or chromanol-alpha-C6 (0.780 μmol/g of tissue) were added to normal skin homogenate (200 mg/ml, 3.2 nmol of endogenous α-tocopherol/g of tissue). Ascorbyl radical was generated from endogenous ascorbate. For incubation and ESR recording conditions see Methods.

To study the interactions of vitamin E with water-soluble antioxidants (ascorbate, reduced thiols) in skin homogenates we used exogenously added α-tocopherol or its homologue with a short (6-carbon) side-chain, chromanol-alpha-C6. The homologue is readily incorporated into membranes and lipid bilayers and gives resolved ESR spectra of its chromanoxyl radicals.[34,35] Addition of exogenous α-tocopherol or chromanol-alpha-C6 to skin homogenate did not affect the endogenous ascorbyl radical ESR signal in the dark. In illuminated samples with added α-tocopherol or chromanol-alpha-C6, the magnitude of the ascorbyl radical signal was initially increased (Figure 6), and a much more rapid decay was observed (Figures 7, 8). In skin homogenates with exogenously added α-tocopherol or chromanol-alpha-C6 the light-induced elimination of the ascorbyl radical signal was accompanied by the appearance of the tocopheroxyl or chomanoxyl radical ESR signals (Figure 6). When exogenous ascorbate was added to α-tocopherol-enriched skin homogenates the length of the lag period during which tocopheroxyl radical ESR signal could not be (or could barely be) detected was greatly increased (from 2 min in the absence of exogenous ascorbate to 16 min in the presence of 1.5 mM ascorbate). The appearance and increase of the tocopheroxyl radical signal occurred only after the gradual decrease and disappearance of the ascorbyl radical signal.

DHLA (3.6 mM) added to α-tocopherol-enriched skin homogenates prior to illumination dramatically increased the persistence of the ESR radical signal of endogenous ascorbate under UV illumination and completely prevented the appearance of the tocopheroxyl radical ESR signal from exogenously added α-tocopherol during a 30 min period (Figure 8). The oxidized form, lipoic acid, was ineffective.

In normal skin homogenates under aerobic conditions, the α-tocopherol concentration decreased from 3.2 nmol/g to 1.5 nmol/g in a non-illuminated sample over 60 min, and to 0.3 nmol/g in an illuminated sample over 60 min. In illuminated skin homogenates with added DHLA (and endogenous ascorbate present), the α-tocopherol

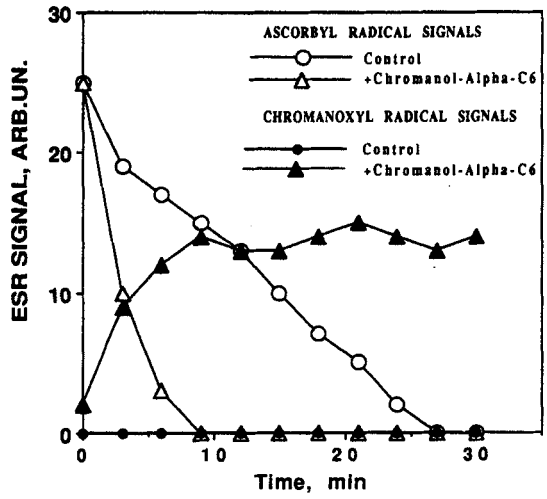

FIGURE 7 Time-course of UV-induced ESR signals of chromanoxyl and ascorbyl radicals in homogenates from skin of hairless SKH-1 mice. Experimental conditions were the same as described in Figure 6. Controls contained only endogenous vitamin E (3.2 nmol of endogenous α-tocopherol/g of tissue); ascorbyl radical was generated from endogenous ascorbate. For incubation and ESR recording conditions see Methods.

concentration was 63% of that in non-illuminated control homogenate after 60 min, whereas in control homogenate with no DHLA added, the concentration of α-tocopherol decreased to 19% of control after 60 min of illumination.

To test whether the photoinduced generation of tocopheroxyl radicals in skin homogenates was due to the direct effect of UVAB absorbed by α-tocopherol we used a set of spectral cut-off filters during illumination of skin homogenates. As in the case of liposomes, filtering out the light in the absorption band of α-tocopherol (λ_{max} 295 nm) decreased the magnitude of tocopheroxyl radicals ESR signal. UV-induced ESR signal of tocopheroxyl radical in skin homogenates was not affected by the cut off filter absorbing in the range up to 264 nm and was completely quenched by the filter absorbing UV up to 315 nm. The filter absorbing UV up to 282 nm decreased ESR signal of tocopheroxyl radical by 15%, while the filter absorbing up to 305 nm quenched the signal by 65% compared to "unfiltered" signal.

These results for skin homogenates are similar to those obtained with liposomes and indicate that the same processes are occurring: direct generation of tocopheroxyl radical by UV light with subsequent depletion of other reducing antioxidants, and sparing of tocopherol if the concentration of other antioxidants is enhanced.

Previous work in our laboratory has demonstrated that cutaneous α-tocopherol can be depleted by illumination with UVB light.[26,27] Since α-tocopherol is considered the major free radical chain-breaking antioxidant in membranes,[24,25] and UV-illumination of skin has been shown to cause lipid peroxidation,[21,23] it is not surprising that α-tocopherol concentrations in skin decrease upon illumination with UV light. However, the present study indicates that α-tocopherol may also be directly destroyed since the tocopheroxyl radical is generated by solar UV light. Dual depletion of this major skin antioxidant implies that, after solar illumination, the skin is highly vulnerable to oxidative stress.

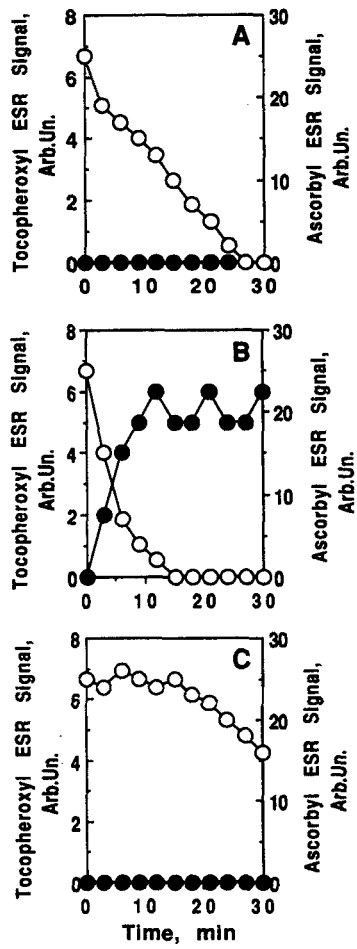

FIGURE 8 Time-course of UV-induced ESR signals of tocopheroxyl and ascorbyl radicals in homogenates from skin of hairless SKH-1 mice. Experimental conditions were the same as described in Figure 6; (---○---) ascorbyl radical signal; (---●---) tocopheroxyl radical signal. (A) Homogenate from normal skin (control); content of endogenous vitamin E (α-tocopherol) was 3.2 nmol/g of tissue; ascorbyl radical was generated from endogenous ascorbate; (B) α-tocopherol was added to control skin homogenate to give final concentration of 780.0 nmol/g of tissue; (C) Dihydrolipoic acid (3.6 mM) was added to a sample identical to the one in B).

Also, because antioxidants like ascorbate and dihydrolipoate can recycle vitamin E, they can become depleted by solar UV illumination. Such depletion leaves the skin more susceptible to free radical attack of vital cellular targets and may actually facilitate tumor initiation and promotion as well as other UV-induced lesions of the skin. The balance between these pro-oxidant and antioxidant roles of vitamin E in skin *in vivo* remains to be elucidated, but it is interesting to note that its endogenous concentration in skin (about 1.0 nmol/g) is much lower than in other tissues which are not exposed to direct UV light.[27,28] However, this concentration can be greatly influenced by topical application of vitamin E, either directly or as a component of skin care

preparations. Further studies are necessary to identify the conditions under which the UV-induced damaging reactions of vitamin E can be minimized compared to its protective radical-scavenging effects. However, these results suggest that bolstering the concentrations of reducing antioxidants capable of recycling vitamin E may be a more effective preventive measure in the skin than directly supplementing vitamin E concentrations. This is consistent with at least one study[39] in which vitamin C was found to have a protective effect against UV light-induced skin cancer in mice, while vitamin E had no effect.

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