Effect of Topically Applied Tocopherol on Ultraviolet Radiation–Mediated Free Radical Damage in Skin

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Previously, we demonstrated by electron paramagnetic resonance (EPR) spectroscopy that ultraviolet radiation induces free-radical formation in Skh-1 hairless mouse skin. Because free-radical oxidative stress is thought to play a principal role in skin photoaging and cancer, oxidative stress and subsequent photodamage should be decreased by supplementation of skin with antioxidants. Using both the ascorbate free radical and an EPR spin-trapping system to detect short-lived radicals, we evaluated the effect of the topically applied antioxidants tocopherol sorbate, α-tocopherol, and tocopherol acetate on ultraviolet radiation–induced free-radical formation. We show that tocopherol sorbate significantly decreases the ultraviolet radiation–induced radical flux in skin. With our chronically exposed mouse model, tocopherol sorbate was also found to be significantly more protective against skin photoaging than α-tocopherol and tocopherol acetate. These results extend our previous observations of ultraviolet radiation–induced free-radical generation in skin and indicate the utility of tocopherol sorbate as an antioxidant in providing significant protection against ultraviolet radiation–induced oxidative damage. ***Key words: antioxidants/ascorbate/electron paramagnetic resonance. J Invest Dermatol 104:484–488, 1995***

Ultraviolet radiation (UV) is thought to produce free-radical species in skin, leading to premature aging and cancer [1–8]. There are several reports providing evidence of this free-radical production in skin [1–2,9–15]. In previous experiments, using room-temperature electron paramagnetic resonance (EPR) spectroscopy, we have detected a very low steady-state level of the ascorbate radical in mouse skin [14,15]. Upon UV radiation exposure of Skh-1 hairless mouse skin the ascorbate free-radical signal intensity increased, indicating free-radical–mediated oxidative stress [15,16]. The ascorbate radical is resonance stabilized and thus easily detected by EPR. However, the non–resonance-stabilized radicals initially produced by UV radiation would have very short lifetimes at room temperature; thus, EPR spin-trapping techniques were used. With these techniques, we previously observed a UV radiation–produced, carbon-centered free-radical spin adduct in skin, characteristic of spin-trapped alkyl radicals generated from membrane lipids [15].

Chronic exposure of skin to UV radiation can significantly decrease cellular and membrane antioxidants [17]; their depletion could lead to unregulated free-radical formation. Therefore, if free radicals are involved, supplementation of skin with antioxidants should prevent radical-mediated oxidative damage. Indeed, Bissett *et al* [18] have shown that application of antioxidants prior to UV exposure delays UV-induced chronic skin damage in hairless mice. Topical and systemic supplementation with tocopherols has been found to be photoprotective by reducing erythema [19–21] as well as by delaying the onset of UV radiation–induced skin-tumor formation during chronic exposure [18,22–25]. Both α-tocopherol and its acetate derivative have been shown to act as UV photoprotectants through antioxidant mechanisms [26]. However, α-tocopherol itself could be photoactive by increasing free radical formation in skin. Tocopherols absorb in the UVB region of sunlight (280–320 nm, $\epsilon 295$ α-tocopherol = 3050 M^{-1} cm^{-1} [27]). UV radiation absorption by α-tocopherol can result in direct conversion to its chromanoxyl radical form [28]. This free-radical form of tocopherol can serve as a pro-oxidant by propagating further deleterious free-radical reactions [29,30], or it may be reduced by other antioxidant systems [31]. This regeneration of α-tocopherol may result in the depletion of other endogenous antioxidants. Consequently, there exists an apparent conflict between the role of tocopherol as a photoprotective antioxidant and as a possible harmful photoreactive agent.

In our study we examined the antioxidant capabilities of α-tocopherol and tocopherol acetate as well as another derivative of vitamin E, tocopherol sorbate. We investigated the UV radiation–induced radicals in intact mouse skin directly by measuring the ascorbate free-radical EPR signal height and indirectly by EPR spin-trapping techniques and examined the effects of these three different chemical forms of vitamin E on reduction or enhancement of UV radiation–induced free-radical production. In addition, we examined the effects of these antioxidants on prevention of skin wrinkling in chronic UVB-exposed mice.

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Abbreviations: $Asc^{\cdot-}$, ascorbate free radical; EPR, electron paramagnetic resonance; G, gauss; IPA, isopropanol; POBN, α-[4-pyridyl 1-oxide]-N-*tert*-butyl nitrone; TOH, tocopherol.

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MATERIALS AND METHODS

Animals Female albino hairless Skh:HR-1 mice (Charles River Laboratories, Portage, MI) were housed and maintained as reported previously [32].

Sample Preparation Mice were topically treated over the dorsal skin with 0.1 ml of vehicle or a tocopherol solution for 3 weeks (three times per week, Monday, Wednesday, and Friday). α-tocopherol acetate and *dl*-α-tocopherol were obtained from Sigma Chemical Co., St. Louis, MO. α-tocopherol sorbate was synthesized as described previously [33]. The test groups, each containing 10 animals, were as follows: isopropanol (IPA) vehicle alone; 5% α-tocopherol in IPA; 5% tocopherol acetate in IPA; and 5% tocopherol sorbate in IPA. The treatments provided approximately a 2 mg/cm^2 coverage of skin, a standard for sunscreen usage in the U.S. [34]. Following 3 weeks of treatment, each group was sacrificed by CO_2 asphyxiation, and the dorsal skins collected. All skin samples were kept at liquid nitrogen temperatures until EPR examination.

Ascorbate Radical Measurement Whole mouse skin was cut into EPR usable pieces (≈1.0 cm^2, epidermis and dermis), placed in a Wilmad Glass Co. (Buena, NJ) tissue cell, and positioned in a TM110 EPR cavity [15]. EPR spectra were obtained at room temperature using a Bruker ESP 300 spectrometer (Bruker Instruments, Karlsruhe, Germany), operated at 9.74 GHz with 100-kHz modulation frequency. The EPR spectrometer settings for the ascorbate radical experiments were microwave power, 40 mW; modulation amplitude, 0.66 G; time constant, 0.3 seconds; scan rate, 8 G/41.9 seconds; and receiver gain, 2×10^6. For both ascorbate and spin-trapping experiments the epidermal surface was exposed to UV radiation while in the EPR cavity, using the same radiation source setup [15].

Spin Trapping For the spin-trapping experiments, α-[4-pyridyl 1-oxide]-N-*tert*-butyl nitrone (POBN) was obtained from Sigma Chemical Co., St. Louis, MO. A 50-μl solution of 250 mM POBN was applied to the epidermis for 9 minutes; the skin was then blotted and placed in a Wilmad tissue cell. Relative radical concentrations were determined by measuring the signal height of the low-field doublet of POBN spin adduct. No significant increase in background EPR signal occurred when POBN alone was exposed to UV radiation. EPR instrument settings for the spin trapping experiments for **Fig 1** were microwave power, 40 mW; modulation amplitude, 0.6 G; time constant, 0.3 seconds; scan rate, 60 G/41.9 seconds; and receiver gain, 1×10^6. The spin-trapping data in **Fig 3** represent four signal-averaged scans where the EPR settings were microwave power, 40 mW; modulation amplitude, 1.06 G; time constant, 0.6 seconds; scan rate, 10 G/41.9 seconds; and receiver gain, 1×10^6.

UV Radiation Setup for EPR The radiation source was a Photomax 150-W xenon arc lamp (Oriel Corporation, Stratford, CT). For the UV radiation experiments wavelengths below 300 nm were filtered out using a 3-mm Schott WG 305 filter (Duryea, PA) (2.9 mW/cm^2 including visible; 1.5 mW/cm^2 for 300–400 nm); for visible-light experiments, wavelengths below 400 nm were filtered out using an Oriel 59472 filter (1.5 mW/cm^2); infrared radiation was removed by a 5-cm water filter. Filtered fluence rates were measured with an International Light (Newburyport, MA) radiometer, assuming that the cavity grid transmits 75% of the incident light.

***In Vivo* Irradiation and Topical Treatment** The procedure for irradiation of the dorsal skin of mice with UVB radiation has been described previously [35]. Briefly, mice (n = 8 per treatment group) were irradiated individually under a bank of four Westinghouse FS-40 sunlamps (UVB radiation, peak emission near 315 nm). Mice were irradiated three times weekly (Monday, Wednesday, and Friday) with 30 mJ/cm^2 UVB radiation per exposure (approximately 0.5 the mouse MED). For topical treatment, the dorsal skin of the mice was treated with 0.1 ml of test solution [isopropanol or 5% (w/v) antioxidant in isopropanol; prepared weekly] 2 h prior to each irradiation.

Skin-Wrinkling Evaluations Skin wrinkling in hairless mice was assessed as described previously [35,36]. The grading scale is 0 to 3, in which 0 is normal and 3 is the maximum visible wrinkle development observed in our work. Visible evaluations were done blind by an individual not involved in the treatment and irradiation work.

Skin lesions were diagnosed and counted as tumors if they were circular, red, raised, and greater than 1 mm in diameter. In other work we have evaluated these types of lesions histologically and found them to be papillomas and squamous cell carcinomas [35].

Statistical Analyses Means at individual time points for treated skin and corresponding vehicle control were calculated. Differences in the mean free-radical production between treatments and vehicle were statistically assessed using a Student t test.

RESULTS

Endogenous Ascorbate-Radical Production The level of free-radical signal in tocopherol-treated mouse skin was examined in room light as well as in the presence of UV radiation. Using room temperature EPR, a very low steady-state level of the ascorbate free radical ($Asc^{\cdot-}$) was observed to be naturally present in skin **(Fig 1**, *top*). Due to ascorbate's role as the terminal small-molecule antioxidant [31,37], the ascorbate-radical concentration can be used as a marker of oxidative stress [16]. Exposure of mouse skin to UV radiation while in the EPR cavity results in an increased $Asc^{\cdot-}$ signal height [15]. This increase indicates that during UV exposure the skin is undergoing free-radical oxidative stress. Exposure to visible light (wavelengths greater than 400 nm) had no affect on $Asc^{\cdot-}$ levels (data not shown).

The tocopherol acetate treatments were found neither to enhance nor protect against UV radiation–induced $Asc^{\cdot-}$ formation **(Fig 2)**. Skin treated with α-tocopherol appears to have increased $Asc^{\cdot-}$ signal in the presence of radiation, indicating that the alpha form of tocopherol could act as a weak photoreactive agent. However, this increase was not found to be significant by statistical analysis. Examining the p values, only tocopherol sorbate treatment was found to provide a significant reduction in $Asc^{\cdot-}$ formation ($p < 0.05$). This reduction was found both in ambient, non-exposed samples as well as in the UV-exposed skin samples.

The ascorbate radical EPR intensity (EPR signal intensity is linearly correlated with steady-state radical concentrations) data were also converted into percentage change versus vehicle control. By taking the difference between control and antioxidant-treated skin $Asc^{\cdot-}$ signal heights at each time point before and after UV exposure, we were able to arrive at overall averages **(Table I)**. Both the baseline and UV radiation–exposed $Asc^{\cdot-}$ intensities for the tocopherol sorbate-treated samples were significantly lower than vehicle values, whereas in the UV radiation–exposed α-tocopherol–treated samples the $Asc^{\cdot-}$ levels were found to be signifi-

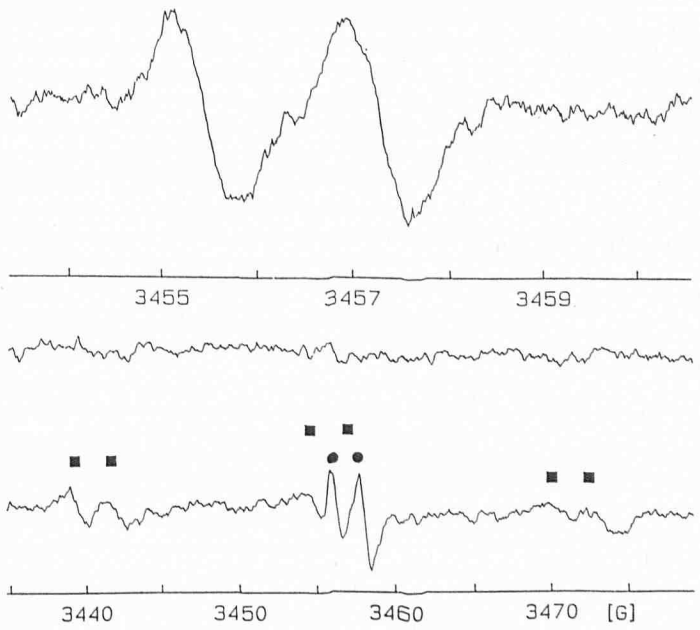

Figure 1. EPR and spin trapping. *Top*, ascorbate radical doublet EPR signal in mouse skin. The EPR doublet signal of $Asc^{\cdot-}$ with hyperfine splitting $a^{H4} \cong 1.8$ G is observed. *Bottom*, POBN spin trapping of a radical from UV radiation–exposed mouse skin. The POBN adduct of a carbon-centered radical (■), $a^N = 15.56$ G, $a^H = 2.70$ G, as well as the $Asc^{\cdot-}$ (●), are shown. The upper spectrum is from skin exposed to room light only, the lower spectrum is from skin exposed to the UV radiation source.

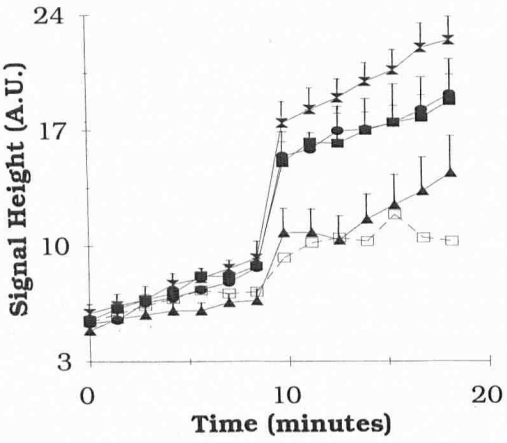

Figure 2. Ascorbate radical EPR signal height decreases in tocopherol sorbate treated skin. □, $Asc^{\cdot-}$ signal height of IPA vehicle-treated skin exposed to room light only; ■, $Asc^{\cdot-}$ signal height of IPA vehicle-treated skin; ●, tocopherol acetate-treated skin; ⧗ α-tocopherol treated skin; and ▲, tocopherol sorbate treated skin during UV exposure. The epidermal surface of the skin was exposed while in the EPR cavity to UV radiation after collection of the seventh data point. Standard error bars were determined for the UV radiation–exposed data. Each point from radiation-exposed samples represents the mean of at least six separate experiments.

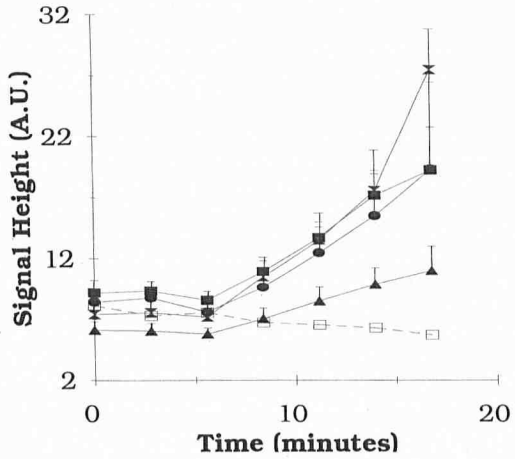

Figure 3. POBN radical adduct EPR signal height decreases in tocopherol sorbate–treated skin. □, POBN radical signal height in IPA vehicle-treated skin exposed to room light only; ■, POBN radical signal height of IPA vehicle-treated skin; ●, tocopherol acetate–treated skin; ⧗, α-tocopherol–treated skin; and ▲, tocopherol sorbate–treated skin during UV exposure. The epidermal surface of the skin was exposed while in the EPR cavity to UV radiation after collection of the third data point. Standard error bars were determined for the UV radiation–exposed data. Each point from radiation-exposed samples represents the mean of at least six separate experiments.

cantly higher than vehicle levels. The acetate form had no effect on $Asc^{\cdot-}$ levels.

UV Radiation–Induced POBN Adduct Signal In the POBN experiments, when skin was exposed to UV radiation both $Asc^{\cdot-}$ as well as a POBN radical adduct signal are detectable by EPR (**Fig 1**, *bottom*). The UV radiation–induced formation of POBN radical adduct was unaffected by the tocopherol acetate treatments (**Fig 3**). The α-tocopherol appears to have enhanced the POBN signal as compared to control during UV exposure; however, this was not found to be significant by statistical analysis. Only the tocopherol sorbate significantly reduced the POBN signal compared to control values.

Each tocopherol treatment time point was compared to vehicle control. Examining the p values for the tocopherol data, only the tocopherol sorbate treatment was found to provide a statistically significant reduction in POBN radical adduct formation. This reduction was found both in baseline samples as well as in the UV-exposed skin samples.

The POBN spin-trapping data were converted into percentage change versus vehicle control as done for the $Asc^{\cdot-}$ data *(vide supra)*, **Table II**. Again, the α-tocopherol and tocopherol acetate forms did not significantly reduce UV-induced radical flux, whereas the tocopherol sorbate treatment was found to dramatically decrease radical levels.

***In Vivo* Photoprotection** The photoprotective effect of topically applied tocopherols against chronic UVB radiation–induced skin damage was evaluated in a mouse model of photoaging [35,36]. In previous testing using this model [18], we observed that tocopherol acetate was poorly photoprotective against chronic UVB radiation–induced skin wrinkling, whereas α-tocopherol provided significant protection. In the present work, we compared the efficacy of these two materials against tocopherol sorbate **(Fig 4)**. The results substantiate our previous observations and indicate that tocopherol sorbate is significantly more protective than the other two forms of vitamin E against skin wrinkling. The study was not continued to the point where all mice had skin tumors, so a thorough evaluation of protection against tumor formation was not possible. However, there were fewer tumors in the tocopherol sorbate group (1.8 tumors/mouse) and α-tocopherol group (2.0 tumors/mouse) versus the vehicle control group (3.6 tumors/mouse) at the end of the study (week 23). Because the tocopherol acetate did not provide significant photoprotection against skin wrinkling at 15 weeks into the study, that group was discontinued prior to the point of first tumor appearance (week 19 in the vehicle control). Whereas tocopherol sorbate and α-tocopherol appeared to reduce the average number of tumors per mouse, they did not delay onset of appearance of the first tumor relative to vehicle.

DISCUSSION

In previous work, using the POBN spin-trapping system, a UV radiation–produced carbon-centered free radical was detected from intact skin [15]. The EPR spectra exhibited hyperfine splittings that are characteristic of POBN/alkyl radicals, possibly generated from membrane lipids as a result of β-scission of lipid alkoxyl radicals.

Table I. Tocopherol Sorbate Reduces Skin Ascorbate Radical: Percentage Change in Skin Ascorbate Radical EPR Signal Height of Tocopherol-Treated Skin Versus Vehicle-Treated Skin[a]

Treatment	Endogenous (before UV)	UV Induced
Alpha tocopherol	+3.7 ± 3.7	+17.6 ± 3.6[b]
Tocopherol acetate	−3.8 ± 5.5	+1.4 ± 2.3
Tocopherol sorbate	−16.2 ± 6.3[b]	−29.1 ± 4.4[b]

[a] Data is presented as mean ± SD.
[b] Significant (at $p < 0.05$) compared to endogenous or UV radiation–exposed vehicle values.

Table II. Tocopherol Sorbate Reduces Skin POBN Radical Adduct: Percentage Change in Skin POBN Radical Adduct EPR Signal Height of Tocopherol-Treated Skin Versus Vehicle-Treated Skin[a]

Treatment	Endogenous (before UV)	UV Induced
Alpha tocopherol	−17.8 ± 2.8[b]	+6.5 ± 2.3
Tocopherol acetate	−8.5 ± 6.4	−11.3 ± 5.0
Tocopherol sorbate	−33.2 ± 2.0[b]	−39.8 ± 11.2[b]

[a] Data is presented as mean ± SD.
[b] Significant (at $p < 0.05$) compared to endogenous or UV radiation–exposed vehicle values.

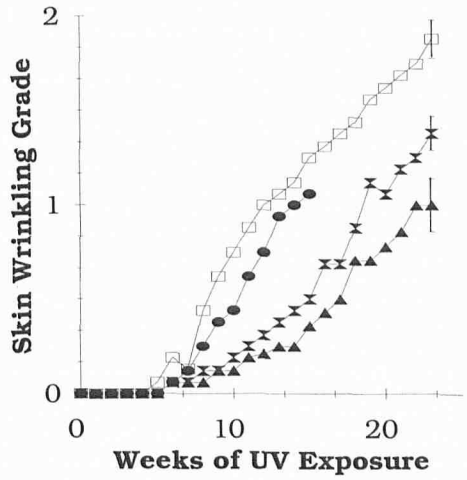

Figure 4. Tocopherols protect against UVB-induced mouse skin wrinkling. Tocopherol acetate, ●, was significantly different from vehicle, □, at only weeks 7–12. Alpha-tocopherol, ⧗, and tocopherol sorbate, ▲, were significantly different from vehicle starting at week 7 and through the remainder of the study.

In lipid peroxidation, propagation is the rate limiting step:

$$LOO\cdot + L'H \rightarrow LOOH + L'\cdot,\ k = 10 \text{ to } 50\ M^{-1}\ \text{second}^{-1}\ [37] \quad (1)$$

where LOO· and L'· are lipid peroxyl and carbon-centered lipid radicals, whereas L'H and LOOH are unsaturated lipid and lipid hydroperoxides. This reaction is slow compared to the addition of oxygen to a lipid radical ($k = 3 \times 10^8\ M^{-1}\ \text{second}^{-1}$ [38]) thus, antioxidants such as tocopherols (TOH) have a chance to compete with oxidizable substrates for the lipid peroxyl radical and thereby break the chain of propagation.

$$LOO\cdot + TOH \rightarrow LOOH + TO\cdot,\ k = 8 \times 10^4\ M^{-1}\ \text{second}^{-1}\ [39]. \quad (2)$$

Thus, by applying TOH and inhibiting the propagation of lipid peroxidation it may be possible to reduce or prevent skin damage associated with free radicals.

α-tocopherol is the active free-radical scavenging form of vitamin E. Norkus *et al* found that topical application of α-tocopherol acetate to mouse skin significantly increases skin α-tocopherol levels, suggesting that α-tocopherol acetate is bioconverted in the skin to free α-tocopherol [40]. In our study, tocopherol sorbate was found to be the most effective in preventing UV-induced skin wrinkling, suggesting that its uptake and appropriate bioconversion is more efficient than the other tocopherol forms examined. Additionally, the sorbate moiety is expected to have an antioxidant activity (e.g., singlet oxygen quenching) due to its conjugated double-bond system [41].

In the skin-wrinkling study α-tocopherol was found to be protective. However, in the EPR experiments, α-tocopherol was found to slightly enhance radical production whereas tocopherol acetate had no effect on radical levels with the methods used. The UV radiation dose used for the EPR free-radical experiments was an acute exposure, whereas the *in vivo* skin-wrinkling experiments involved chronic UV exposure. The increased free-radical levels in the acute-exposure experiments could be due to α-tocopherol absorbing UV radiation itself and being directly converted to its free-radical form.

The tocopherol sorbate treatment was found to be highly photoprotective against UV radiation–induced free-radical formation and photoaging in the hairless mouse model. There was almost 50% less detectable radical formation in UV radiation–exposed tocopherol sorbate treated than in α-tocopherol–treated skin. In addition, tocopherol sorbate treatment decreased baseline radical formation in skin, suggesting a possible anti-aging role for this treatment. Further work is needed to elucidate the mechanism involved in protection.

The use of topical antioxidants to reduce free-radical formation is a promising new approach to photoprotection and possibly skin-tumor prevention. In addition to blocking the UV radiation from being absorbed by skin, as is typically done with UV absorbers, such as *para*-aminobenzoic acid and cinnamate (sunscreen ingredients), skin could also be protected by preventing propagation of free-radical damage.

Currently, sunscreens and many cosmetics contain tocopherol in the acetate form which, based on the methods used here, has only a modest protective effect. Our data clearly demonstrate that the sorbate form of vitamin E significantly reduces the level of free radicals in UV radiation–exposed mouse skin. This reduction in measured free-radical signal in tocopherol-treated skin correlates with a decrease in photo-induced skin wrinkling in animals similarly treated, suggesting a connection between free radicals and wrinkling. Thus, our data support the use of tocopherol sorbate in sunscreen preparations to reduce photoaging.

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REFERENCES

1. Norins AL: Free radical formation in the skin following exposure to ultraviolet light. *J Invest Dermatol* 39:445–448, 1962
2. Pathak MA, Stratton K: Free radicals in human skin before and after exposure to light. *Arch Biochem Biophys* 123:468–476, 1968
3. Black HS: Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochem Photobiol* 46:213–221, 1987
4. Fuchs J, Mehlhorn RJ, Packer L: Free radical reduction mechanisms in mouse epidermis skin homogenates. *J Invest Dermatol* 93:633–640, 1989
5. Schallreuter KU, Wood JM: Free radical reduction in the human epidermis. *Free Radic Biol Med* 6:519–532, 1989
6. Meffert H, Diezel W, Sonnichsen N: Stable lipid peroxidation products in human skin: detection, ultraviolet light-induced increase, pathogenic importance. *Experientia* 32:1397–1398, 1976
7. Miyachi Y, Toda K, Nishigori C, Imamura S: Reactive oxygen species in cutaneous photobiology. *Photomed Photobiol* 11:71–79, 1989
8. Darr D, Fridovich I: Free radicals in cutaneous biology. *J Invest Dermatol* 102:671–675, 1994
9. Nishi J, Ogura R, Sugiyama M, Hidaka T, Kohno M: Involvement of active oxygen in lipid peroxide radical reaction of epidermal homogenate following ultraviolet light exposure. *J Invest Dermatol* 97:115–119, 1991
10. Ogura R, Sugiyama M, Nishi J, Haramaki N: Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J Invest Dermatol* 97:1044–1047, 1991
11. Ogura R, Sugiyama M, Sakanashi T, Hidaka T: Role of oxygen in lipid peroxide formation of skin exposed to ultraviolet light. In: Hayaishi O, Imamura S, Miyachi Y (eds.). *The Biological Role of Reactive Oxygen Species in Skin*. Elsevier, New York, pp 55–63, 1987
12. Taira J, Mimura K, Yoneya T, Hagi A, Murakami A, Makino K: Hydroxyl radical formation by UV-irradiated epidermal cells. *J Biochem* 111:693–695, 1992
13. Sugiyama M, Kaneko N, Kagiyama A, Morakata S, Ogura R: Lipid peroxide and free radical formation in the epidermis following single exposure of ultraviolet light. *J Kurume Med Assoc* 47:346–350, 1984
14. Buettner GR, Motten AG, Hall RD, Chignell CF: ESR detection of endogenous ascorbate free radical in mouse skin: enhancement of radical production during UV irradiation following topical application of chlorpromazine. *Photochem Photobiol* 46:161–164, 1987
15. Jurkiewicz BA, Buettner GR: Ultraviolet light-induced free radical formation in skin. *Photochem Photobiol* 59:1–4, 1994
16. Buettner GR, Jurkiewicz BA: Ascorbate free radical as a marker of oxidative stress: an EPR study. *Free Radic Biol Med* 14:49–55, 1993
17. Maeda K, Naganuma M, Fukuda M: Effects of chronic exposure to ultraviolet-A including 2% ultraviolet-B on free radical reduction systems in hairless mice. *Photochem Photobiol* 54:737–740, 1991
18. Bissett DL, Chatterjee R, Hannon DP: Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in hairless mouse. *Photodermatol Photoimmunol Photomed* 7:56–62, 1990
19. Trevithick JR, Xiong H, Lee S, Shun DT, Sanford SE, Karlik SJ, Norley C, Dilworth GR: Topical tocopherol acetate reduces post-UVB, sunburn-associated erythema, edema and skin sensitivity in hairless mice. *Arch Biochem Biophys* 269:575–582, 1992
20. Roshchupkin D, Pistsov M, Potapenko A: Inhibition of ultraviolet light-induced erythema by antioxidants. *Arch Dermatol Res* 266:91–94, 1979
21. Fryer M: Evidence for the photoprotective effects of vitamin E. *Photochem Photobiol* 58, 304–312, 1993

22. Bissett DL, Hillebrand GG, Hannon DP: The hairless mouse as a model of skin photoaging: its use to evaluate photoprotective materials. *Photodermatol* 6:228–233, 1989
23. Bissett DL, Chatterjee R, Hannon DP: Protective effects of a topically applied antioxidant plus an antiinflammatory agent against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *J Soc Cosmet Chem* 43:85–92, 1992
24. Black HS, Lenger W, McCann V, Thronby J: Relation of UV dose to antioxidant modification of photocarcinogenesis. *J Am Coll Toxicol* 2:201–207, 1983
25. Black HS, Chan JT: Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. *J Invest Dermatol* 65:412–414, 1975
26. Beijersbergen van Henegouwen GMJ, de Vries H, van den Broeke LT, Junginger HE: RRR-Tocopherols and their acetates as a possible scavenger of free radicals produced in the skin upon UVA-exposure—an in vivo screening method. *Fat Sci Technol* 94:24–27, 1992
27. Windholz M. Tocopherol. In: *The Merck Index, 10th ed.* Merck & Co., Rahway, NJ, 1983
28. Kagan V, Witt E, Goldman G, Scita G, Packer L: Ultraviolet light-induced generation of vitamin E radicals and their recycling. A possible photosensitizing effect of vitamin E in skin. *Free Radical Res Commun* 16:51–64, 1992
29. Bowry VW, Stocker R: Tocopherol-mediated peroxidation. The prooxidant effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein. *J Am Chem Soc* 115:6029–6044, 1993
30. Mukai K, Sawada K, Kohno Y, Terao J: Kinetic study of the prooxidant effect of tocopherol: hydrogen abstraction from lipid hydroperoxides by tocopheroxyls. *Lipids* 28:747–752, 1993
31. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 86:6377–6381, 1989
32. Bissett DL, Oelrich DM, Hannon DP: Evaluation of a topical iron chelator in animals and in human beings: Short-term photoprotection by 2-furildioxime. *J Am Acad Dermatol* 31:572–578, 1994
33. Bissett DL, Bush RD: Photoprotection compositions comprising tocopherol sorbate. *U.S. Patent* 4,847,072, 1989
34. Food and Drug Administration: Sunscreen drug products for over-the-counter drugs. *Fed Reg* 43:38259, 1978
35. Bissett DL, Chatterjee R, Hannon DP: Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol Photoimmunol Photomed* 7:153–158, 1990
36. Bissett DL, Hannon DP, Orr TV: An animal model of solar-aged skin: histological, physical, and visible changes in UV-irradiated hairless mouse skin. *Photochem Photobiol* 46:367–378, 1987
37. Buettner GR: The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys* 300:535–543, 1993
38. Hasegawa K, Patterson LK: Pulse radiolysis studies in model lipid systems: formation and behavior of peroxy radicals in fatty acids. *Photochem Photobiol* 28:817–823, 1978
39. Patterson LK: In: Rodgers MAJ, Powers EL (eds.). Oxygen and Oxy-Radicals in Chemistry and Biology. Academic Press, New York, 1981
40. Norkus EP, Bryce GF, Bhagavan HN: Uptake and bioconversion of α-tocopherol acetate to α-tocopherol in skin of hairless mice. *Photochem Photobiol* 57:613–615, 1993
41. Bissett DL, Majeti S, Fu J-JL, McBride JF, Wyder WE: Protective effect of topically applied conjugated hexadienes against ultraviolet radiation–induced chronic skin damage in the hairless mouse. *Photodermatol Photoimmunol Photomed* 7:63–67, 1990