

REVIEW

The role of antioxidants in photoprotection: A critical review

Lucy Chen, BA,^a Judy Y. Hu, MD,^b and Steven Q. Wang, MD^a
New York, New York, and Hermitage, Tennessee

Free radicals have long been studied as a contributor to aging and disease processes. Endogenous production of radicals from cellular metabolism and exogenous sources from ultraviolet radiation and pollution can damage the skin on the cellular and tissue levels. Although the body possesses an elegant defense system to prevent radical damage, this innate system can be overwhelmed and lead to a state of oxidative stress or immunosuppression, and can even trigger carcinogenesis. Topical supplementation of antioxidants can provide additional protection to neutralize reactive oxygen species from both endogenous and exogenous sources. This review will discuss our current understanding of the mechanisms of free radical damage and evaluate the potential benefit of topical antioxidants in sunscreens and skin care products. (J Am Acad Dermatol 10.1016/j.jaad.2012.02.009.)

Key words: antioxidants; free radicals; photoaging; photoprotection; reactive oxygen species; sunscreen.

Overexposure to ultraviolet (UV) radiation (UVR) from the sun plays an important role in the development of skin cancers and skin aging. Over the past decade, there has been an increasing understanding on the mechanism by which UVA damages the skin. This awareness is reflected in the development of newer sunscreen formulations with protection extending to the long range of UVA wavelengths. This insight, combined with the knowledge that UVA induces free radicals, has led to a renewed research focus on the detrimental role of free radicals on skin health. Although the body has an innate antioxidant (AOx) defense system to neutralize these radicals generated from both the exogenous and endogenous sources, this AOx reservoir can be quickly depleted. Hence, topical supplementation of AOxs, at least in theory, holds the promise of providing extra benefit to the skin, especially under oxidative stress from excessive amount of UVA exposure.

In this review, we will discuss the sources of free radicals, explain the mechanisms of damage from these radicals, and highlight the cellular and clinical consequences. In addition, we will review common AOxs with demonstrated benefits. Lastly, we will

Abbreviations used:

AOx:	antioxidant
AP-1:	activation protein-1
ATP:	adenosine triphosphate
GSH:	glutathione
H ₂ O ₂ :	hydrogen peroxide
LC:	Langerhans cell
MMP:	matrix metalloproteinase
NF-κB:	nuclear factor-κB
O ₂ ^{•-} :	superoxide anion
OH•:	hydroxyl radical
ROS:	reactive oxygen species
SOD:	superoxide dismutase
UV:	ultraviolet
UVR:	ultraviolet radiation
¹ O ₂ :	singlet oxygen

examine the limitations in formulating sunscreen and skin care formulations with active AOxs.

PART I: FREE RADICALS

A free radical is defined as a species that can exist independently with one or more unpaired electrons.¹ In living systems, free radicals are predominantly represented as reactive oxygen species (ROS), taking form as oxygen-centered oxidizing agents.

From the Dermatology Service, Memorial Sloan-Kettering Cancer Center, New York,^a and Cumberland Skin Surgery and Dermatology, Hermitage.^b

Funding sources: None.

Disclosure: Dr Wang is a consultant for L'Oreal. Ms Chen and Dr Hu have no conflicts of interest to declare.

Reprint requests: Steven Q. Wang, MD, Dermatology Service, Memorial Sloan-Kettering Cancer Center, 160 E 53 St, New York, NY 10022. E-mail: wangs@mskcc.org.

Published online March 9, 2012.

0190-9622/\$36.00

© 2012 by the American Academy of Dermatology, Inc.

doi:10.1016/j.jaad.2012.02.009

The most common oxygen-based ROS are: superoxide anion ($O_2^{\bullet-}$), peroxide, hydroxyl radical ($OH\bullet$), hydroxyl ion, and singlet oxygen (1O_2), an excited state of molecular oxygen. ROS are volatile and unstable. In biological systems, ROS add electrons (oxidize) to other nearby molecules to release the extra energy and return to stable states. When not quenched by AOxs, the oxidation reactions can continue, or unravel into cascades with damaging consequences.

A significant source of endogenous ROS comes from the byproduct of oxidative metabolism in the mitochondria where adenosine triphosphate (ATP) is generated from glucose.² In a coordinated reaction, electrons pass through 4 complexes of the electron transport chain to generate ATP and water (Fig 1). As a side reaction,

molecular oxygen is also converted to $O_2^{\bullet-}$, a volatile and potent ROS.³ It is estimated that 1% to 2% of the oxygen present in the cell divert to these side reactions.⁴ Aside from the ATP-generation process, $O_2^{\bullet-}$ can also be generated by xanthine oxidase for the degradation of purine nucleotides and by nitric oxide synthase for the production of nitric oxide, a secondary messenger. $O_2^{\bullet-}$ is converted into hydrogen peroxide (H_2O_2) by spontaneous conversion or superoxide dismutase (SOD) (Fig 2). H_2O_2 is the key agent in the Fenton reaction, which readily occurs in the presence of metal catalysts (iron or copper) and produces $OH\bullet$, one of the most unstable ROS that exists in a biological system.⁵ The half-life of $OH\bullet$ is so short (10^{-9} seconds) that it can exert its damaging effects at nearly exclusively the site of its generation.⁶

Exogenous ROS production comes from environmental sources such as UVR, pollutants, and xenobiotics (Fig 3). Measurable levels of H_2O_2 and $OH\bullet$ occur within 15 minutes after UV exposure and continue for up to 60 minutes.^{7,8} The action spectrum for ROS generation is predominately in the UVA range (320–400 nm), although there is some overlap with UVB.⁹ UVA reacts with photosensitizers or chromophores in the skin, such as cytochromes, riboflavin, heme, and porphyrin. These chromophores absorb the energy from the UVA wavelength and transition into an excited, unstable state. The energy expelled upon return to the stable state is transferred to nearby oxygen molecules to generate 1O_2 and other ROS.^{10,11} Collectively, these ROS can

cause nonspecific cellular damage to DNA, protein, and lipid structures. Environmental pollutants such as polycyclic aromatic hydrocarbons from fossil fuel combustion can be activated and converted into endogenous ROS via quinone intermediates.¹² In vitro and in vivo studies demonstrate that a common polycyclic aromatic hydrocarbons, benzo-

apryrene and its intermediates, act as photosensitizers, which upon UVA exposure, synergistically increase production of superoxide and 1O_2 .^{12–16}

CELLULAR DAMAGE FROM FREE RADICALS

Exposure to excess UV irradiation and pollutants leads to a pro-oxidant state. The resulting oxidative stress can impact the genetic integrity of a living organism. Whereas UVB directly damages DNA, UVA acts by ROS

intermediates. ROS-induced DNA damages can lead to the formation of a modified guanine nucleotide (8-hydroxyguanine), single-stranded breaks, and oxidized pyrimidine bases.^{17,18} These damages, although predominantly UVA related, have been observed in UVB-irradiated cells.¹⁹ Incorporation of 8-hydroxyguanine into DNA strands has been implicated in tumor promotion, suggesting that permanent DNA damage leads to mutagenesis and carcinogenesis.^{20,21} In addition to nuclear DNA, the 4977-base pair mitochondrial DNA deletion, known as the “common deletion,” is prevalent in human skin irradiated with UVA.²² The mechanism has been attributed to the generation of 1O_2 .²³

Cellular phospholipid membranes and proteins are also targets of oxidative reactions incurred by UV rays and ROS. Lipid peroxidation is initiated by an unstable $OH\bullet$ that abstracts a hydrogen atom from nearby unsaturated fatty acid. This forms lipid molecules with extra electrons, which form peroxy radicals in the presence of molecular oxygen. If not quickly terminated, a chain reaction can occur, wreaking havoc on neighboring lipids and disintegrating the cell membrane. Oxidative damage at the protein level is reflected in modification of the polypeptide chain to form carbonyl derivatives. Protein oxidation products appear to accumulate and persist preferentially in the dermis.²⁴ As DNA, lipid, and protein damages accrue in a cell undergoing oxidative stress, events can potentially spiral toward apoptosis. The role of 1O_2 and $O_2^{\bullet-}$ in

CAPSULE SUMMARY

- Free radicals from endogenous and exogenous sources can damage DNA, lipid membrane, and protein structures, and can also induce photocarcinogenesis and photoaging.
- Topical antioxidants have the potential to supplement the body's innate defense to neutralize free radicals.
- Challenges remain in effectively incorporating antioxidants into sunscreens and skin care products.

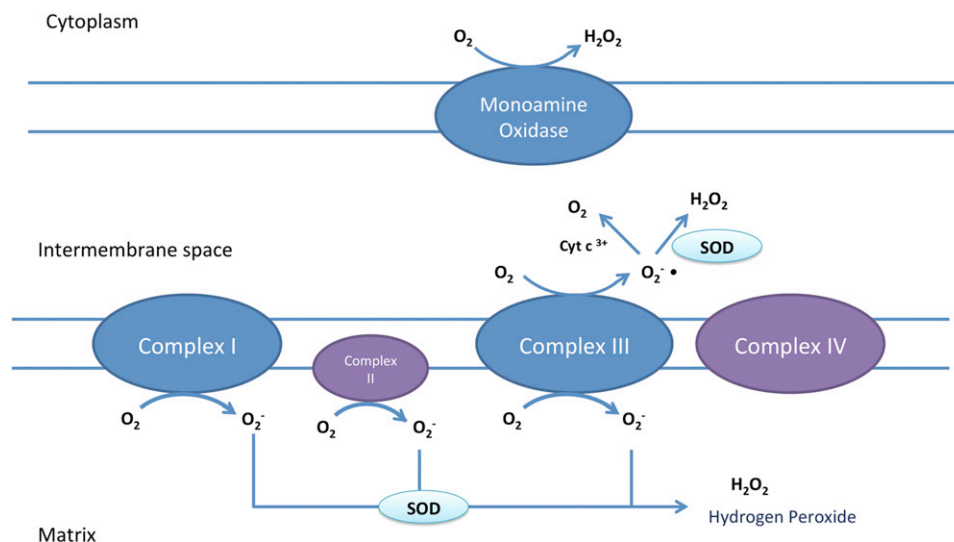


Fig 1. Formation of superoxide in mitochondrial respiratory chain. Complexes in mitochondrial respiratory chain leak electrons to oxygen-producing superoxide anion ($O_2^{\bullet-}$). Increased concentrations of $O_2^{\bullet-}$ may reduce transition metals, which in turn react with hydrogen peroxide (H_2O_2)-producing hydroxyl radicals (OH^{\bullet}) or may react with nitric oxide to form peroxynitrite. Both OH^{\bullet} and peroxynitrite are strong oxidants that indiscriminately react with DNA, lipids, and proteins. $O_2^{\bullet-}$ can be converted into H_2O_2 and oxygen in both intermembrane space and matrix of mitochondria. Reprinted with permission from Turrens.¹¹⁴ *Cyt c*, Cytochrome c; *SOD*, superoxide dismutase.

apoptosis has been demonstrated in cell culture studies.²⁵

CUTANEOUS DAMAGE FROM FREE RADICALS

Photoaging

Harman²⁶ first proposed the free radical theory of aging in 1956 stating that free radical accumulation was contributing to the cumulative changes seen in aging. Indeed, free radical damage on the skin by chronic ROS and UV stress plays a major role in photoaging (Fig 4). After UV exposure, ROS trigger the release of proinflammatory cytokines and growth factors.^{8,27} Specifically, factors activation protein-1 (AP-1) and nuclear factor- κ B (NF- κ B) up-regulate key matrix metalloproteinases (MMP) such as MMP-1, MMP-3, MMP-8, and MMP-9. Collectively, these proteases degrade the collagen and elastin fibers of the extracellular matrix.²⁸ Interestingly, MMP-1 expression is associated with the presence of mitochondrial DNA common deletion, reinforcing the possibility that ROS affects many points along this pathway.^{29,30} Furthermore, UVR-induced ROS have been shown to decrease transforming growth factor- β expression, which decreases collagen production and enhances elastin production.³¹⁻³³ Hence, ROS degrade the structural integrity of skin by way of altering the collagen and elastin components of the extracellular matrix.

Immunosuppression

It is known that both UVA and UVB can initiate immunosuppression of the skin.³⁴ The mechanism of UVA immunosuppression is not completely known but a ROS-dependent mechanism has been implicated. UVA-induced ROS can lead to lipid peroxidation, disturb redox potential, initiate AP-1 and NF- κ B transcription, and eventually activate downstream cytokines (interleukin-4 and -10), which are responsible for systemic immunosuppression.^{35,36} Mechanistic studies using sunscreens and AOxs specifically implicate ROS in UV-induced immunosuppression, measured by depletion of epidermal Langerhans cells (LC) and suppression of contact hypersensitivity in skin studies.³⁷ With the application of sunscreen, depletion of epidermal LC is prevented and delayed hypersensitivity is improved. The degree of protection is directly related to the level of UVA protection.³⁸⁻⁴¹ In mice studies, Halliday et al⁴² used AOxs to evaluate UVA-induced immunosuppression. In the presence of topical L-NMMA (nitric oxide inhibitor), iron chelator 2,2'-dipyridyl, and the SOD-mimicking agent 4-hydroxytempol, antigen induction on irradiated skin was reduced to undetectable levels. Similar results using biologically active AOxs, such as green tea polyphenols, have shown a reduction in markers of immunosuppression.⁴³⁻⁴⁵ In human studies, application of a formulation of topical AOxs, even in the absence of

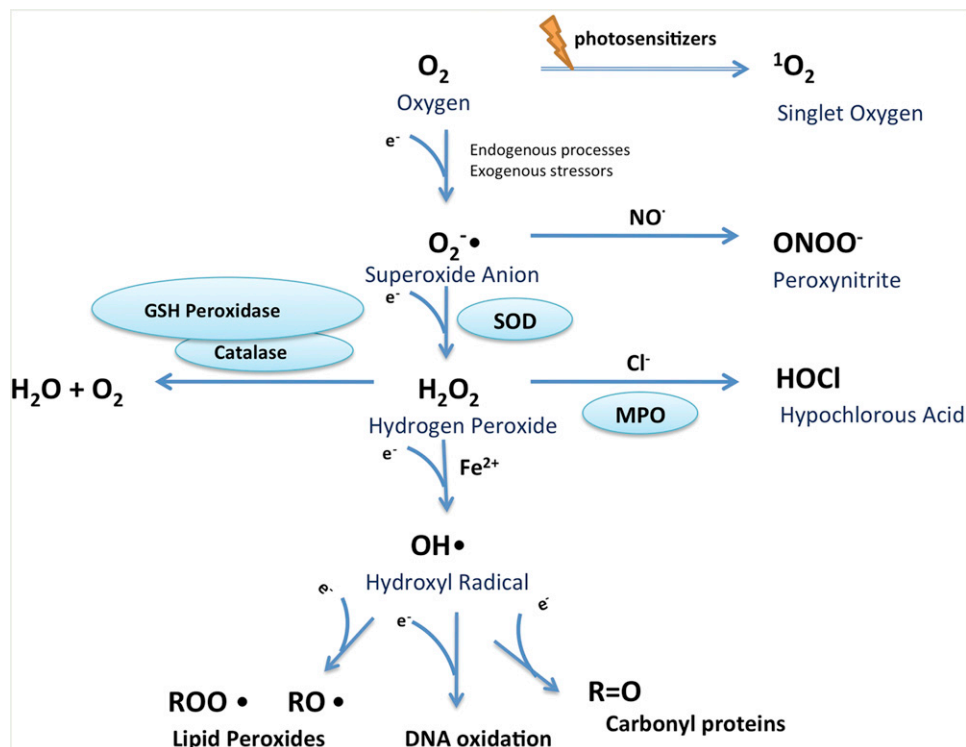


Fig 2. Generation of reactive oxygen species (ROS). Oxygen molecule can be converted into singlet oxygen (1O_2) or superoxide anion ($O_2^{\bullet-}$). $O_2^{\bullet-}$ is extremely unstable and can be further converted to hydrogen peroxide (H_2O_2) either spontaneously or enzymatically by superoxide dismutase (SOD). H_2O_2 is more stable than $O_2^{\bullet-}$ and can permeate through lipid membrane of cells. ROS can be neutralized to form water and oxygen or hypochlorous acid. H_2O_2 can also be converted to hydroxyl radical (OH^{\bullet}) in presence of iron (Fe^{2+}) via Fenton reaction (ie, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + \text{hydroxyl ion}$). OH^{\bullet} can react with nucleotides, unsaturated lipids, and amino acids or be neutralized to water. *GSH*, Glutathione; O_2 , molecular oxygen.

sunscreen, can also prevent LC depletion.⁴⁶ Considerably more mechanistic work needs to be done in this field to determine the role of ROS in immunosuppression.

Photocarcinogenesis

Although the relationship between UVR and photoaging is well described, the mechanistic connection between ROS and skin cancer is still unclear. At the molecular level, it has been demonstrated that ROS interfere with normal cell signaling by affecting expression of signal transduction genes.⁴⁷ Aberrant AP-1 and NF- κ B pathways have been implicated in cell proliferation and apoptosis leading to carcinogenesis. Halliday³⁴ examined DNA from human actinic keratoses and squamous cell carcinomas for signature ROS mutations. A large number of mutations in both groups were found to be ROS induced on the p53 gene, suggesting that ROS can be a mutagen, driving precursor lesions to malignancy. In addition, the presence of topical AOXs and ROS

inhibitors reduced UV-induced skin carcinogenesis in mice, suggesting a method to attenuate carcinogenesis by reducing ROS.^{42,48,49}

INNATE DEFENSE SYSTEM AGAINST FREE RADICALS

Human skin has an elaborate enzymatic and nonenzymatic AOX defense network against ROS (Table I). The key AOX enzymes include SOD, catalase, and glutathione (GSH) peroxidase. SOD catalyzes the conversion of two volatile superoxide radicals into less volatile H_2O_2 and oxygen. H_2O_2 is further reduced to water and oxygen with the aid of catalase and GSH peroxidase (Fig 2). The nonenzymatic AOXs can occupy lipid- and water-soluble compartments of the cell, and the concentration and activity levels of these AOXs are higher in the epidermis than dermis. Both the enzymatic and nonenzymatic AOXs work in a coordinated fashion to neutralize ROS. For example, GSH reductase can regenerate GSH from GSH disulfide, the oxidized

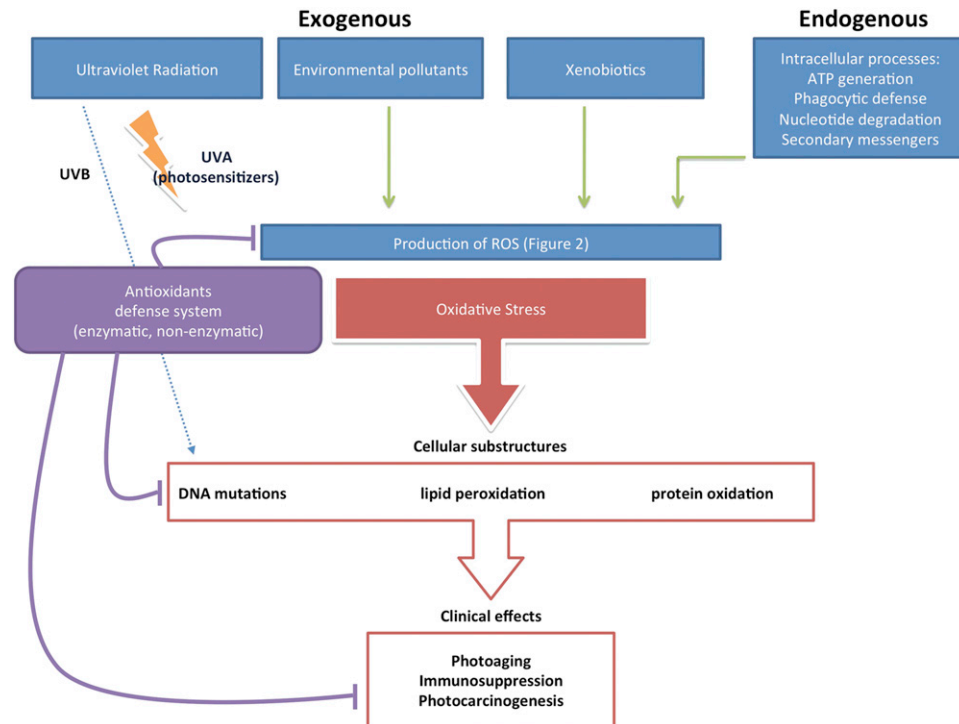


Fig 3. Cellular and clinical effects of reactive oxygen species (ROS). ROS are generated from exogenous and endogenous sources. On cellular level, ROS has potential to cause DNA mutation, lipid peroxidation, and protein oxidation. On clinical level, ROS plays a role in photoaging, immunosuppression, and photocarcinogenesis. Antioxidants maintain redox state by quelling these harmful ROS. UV, Ultraviolet.

form of GSH. In turn, GSH can restore vitamins C and E from the oxidized to the reduced state, thereby activating these two AOX to neutralize additional ROS. At the molecular level, another key mechanism against oxidative damage is the transcription factor, NF-E2-related factor 2 (Nrf2), and its transcriptional activation of AOX enzymes. Most recent studies have demonstrated that Nrf2 is protective of both skin keratinocytes and fibroblasts against UVA-oxidative damage.^{50,51} This may be a promising field for therapeutic applications targeting the innate defenses of AOX.

Despite these innate defenses, increased oxidative stress can overwhelm the skin's AOX reserves and enzymatic machinery. Shindo et al^{52,53} demonstrated decreased levels of both enzymatic (SOD, GSH peroxidase, catalase activity) and nonenzymatic (α -tocopherol, GSH, and L-ascorbic acid) AOXs on mice skin when the animals were exposed to acute UV irradiation. In human beings, even at suberythrogenic UVR doses, the AOXs in the stratum corneum are susceptible to depletion.⁵⁴ Aging also diminishes AOX levels: compared with young human subjects, elderly subjects had 70% less concentration of α -tocopherol, L-ascorbic acid, and total GSH in their skin.⁵⁵

PART II: TOPICAL ANTIOXIDANTS

There is a growing trend in incorporating AOXs in sunscreens and skin care products to replenish the natural reservoirs in the skin. Topical AOXs have the potential to diminish the ROS generated from the UVA radiation. In the following section, common topical AOXs and their effectiveness as a component of photoprotection are reviewed, and additional compounds with AOX properties are featured in Table II.

Vitamin C

Vitamin C is a water-soluble AOX and it is the predominant AOX in the skin based on molar concentrations.⁵⁴ Vitamin C neutralizes free radicals in aqueous compartments of the skin, and also plays a role in regenerating vitamin E. Aside from serving as an AOX, it is also a cofactor for critical enzymes in collagen synthesis and can inhibit elastin biosynthesis to reduce elastin accumulation.⁵⁶ It also reduces pigment darkening by inhibiting tyrosinase and maintains hydration by protecting the epidermal barrier of the skin.⁵⁷ At the molecular level, addition of topical 1% vitamin C increases collagen synthesis and reduces MMP (collagenase) expression.⁵⁸

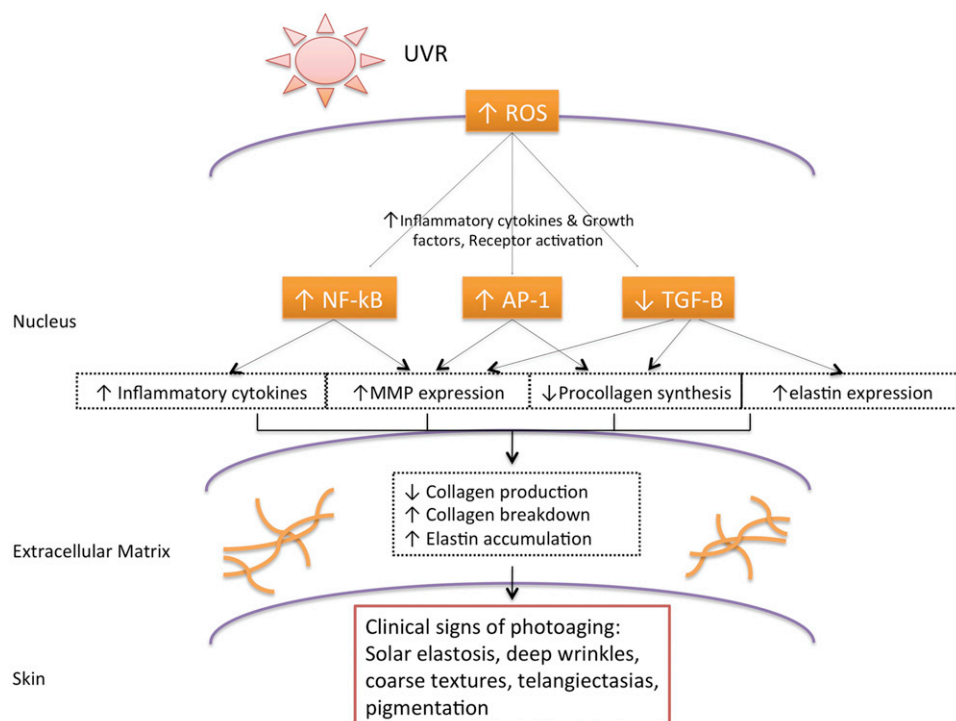


Fig 4. Role of reactive oxygen species (ROS) in photoaging. ROS from exogenous (eg, ultraviolet [UV] radiation) and endogenous sources initiates signal transduction cascade resulting in up-regulation of AP-1, NF-kB, and down-regulation of transforming growth factor (*TGF*)- β . Downstream, NF-kB signals increase in interleukin-1 and tumor necrosis factor- α levels, and AP-1 activates matrix metalloproteinases (*MMP*). Decrease in *TGF*- β expression leads to decrease in collagen synthesis. Cumulatively, these changes lead to increase in collagen breakdown, and increase in elastin production in extracellular matrix.

Table I. Endogenous antioxidants

Nonenzymatic antioxidants	
α -Tocopherol (vitamin E)	
Ascorbic acid (vitamin C)	
Glutathione	
Carotenoids	
Ubiquinone	
Flavonoids	
Uric acid	
Enzymatic antioxidants	
Superoxide dismutase	
Glutathione peroxidase	
Glutathione reductase	
Catalase	

Application of topical L-ascorbic acid has been shown to have photoprotective effects including the reduction of erythema,⁵⁹ sunburn cell formation,⁵⁹ and immunosuppression.⁶⁰

Delivery of topical application into the skin is a challenge. To penetrate the stratum corneum, L-ascorbic acid must lose its ionic charge and be in a formulation with a pH less than 3.5. At these pH settings, the hydroxyl group of L-ascorbic acid is

unstable. As a result, many formulators use more stable esterified substitutes, such as magnesium ascorbyl phosphate and ascorbyl-6-palmitate. Compared to L-ascorbic acid, the AOx activities of these substitutes are inferior and do not achieve the same activity levels in vivo.^{57,61,62}

Vitamin E

Vitamin E is a lipid-soluble AOx, and it exists as 8 major compounds (4 tocopherols and 4 tocotrienols) with the most abundant form being α -tocopherol. Its main function is to protect the cell membranes from oxidative stress. The highest concentration of vitamin E is delivered to the deepest layers of the stratum corneum by sebaceous gland secretion. The level of vitamin E can be depleted even after a single suberythemogenic dose of UVR exposure.⁵⁴

A multitude of animal and human studies have demonstrated a reduction in lipid peroxidation,⁶³ photoaging,^{64,65} immunosuppression,^{48,66,67} and photocarcinogenesis^{48,49} after topical vitamin E application. On the molecular level, topical α -tocopherol decreases MMP-1 transcription levels and

Table II. Benefits of antioxidants in topical formulation

Antioxidant compound	Sources	Clinical end points studied
Vitamin C (ascorbyl palmitate, magnesium, ascorbyl phosphate)	Fruits, vegetables	Erythema ⁵⁹ Immunosuppression ⁶⁰ Photoaging ⁹² Photocarcinogenesis ⁶⁴
Vitamin E (α -tocopherol acetate, α -tocopherol succinate)	Vegetable oil, seeds, nuts, meats	Erythema ^{93,94} Photoaging ^{64,65} Immunosuppression ^{48,66} Photocarcinogenesis ^{48,49}
Vitamin A (retinols, carotenoids)	Colored fruits and vegetables (eg, tomatoes, sweet potatoes)	Photoaging ⁹⁵
Selenium	Corn, wheat, soybean	Erythema ^{77,96} Photocarcinogenesis ^{78,96}
Silymarin	Milk thistle	Photocarcinogenesis ^{80,97} Immunosuppression ⁹⁸
Green tea polyphenols (epicatechin, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate)	Fractions isolated from tea	Erythema ⁴⁴ Immunosuppression ⁴³⁻⁴⁵ Photoaging ⁹⁹ Photocarcinogenesis ⁸³
Soy isoflavones (genistein, daidzein, equol)	Soy, red clover, ginkgo biloba	Erythema ^{84,100,110} Photoaging ^{84,102} Immunosuppression ¹⁰¹ Photocarcinogenesis ^{84,103}
Caffeic acid (ferulic acid, caffeic acid phenethyl ester)	Coffee beans, propolis, plant seeds	Erythema ¹⁰⁴ Immunosuppression ¹⁰⁵
Apigenin	Fruits and leafy vegetables, tea, wine	Photoaging ¹⁰⁶ Photocarcinogenesis ¹⁰⁷
Polypodium leucotomos extract	Tropical fern plant Polypodium leucotomos	Erythema ¹⁰⁸ Photoaging ^{109,110} Photocarcinogenesis ¹⁰⁹
Pycnogenol	Extract from bark of maritime pine tree	Inflammation ¹¹¹ Immunosuppression ¹¹¹ Photocarcinogenesis ¹¹¹
Resveratrol	Skin and seeds of grapes, nuts, fruits, red wine	Erythema ¹¹² Photocarcinogenesis ¹¹³

inhibits thymine dimer formation, thereby slowing down the process of collagen breakdown and mutagenesis, respectively.^{68,69} The protection against dimer formation has been postulated to be a result of the AOx interplay with ROS rather than a UVB-absorbing sunscreen effect.⁷⁰

Vitamins C and E work in conjunction in an elaborate network of redox reactions to stave off oxidative stress. Vitamin C regenerates oxidized vitamin E at sites of lipid peroxidation. Oxidized vitamin C requires GSH for its own regeneration. This interaction maintains the AOx reservoir in the skin tissues. Compared with vitamin C alone, the combination of 15% L-ascorbic acid and 1% α -tocopherol doubles the protection against UV-induced erythema, sunburn cell formation, and thymine dimer formation.⁷¹ Moreover, stabilizing agents such as 1.5% ferulic acid and phloretin, two powerful plant AOxs, provide even greater benefit in vitamin C

and E combination formulas, possibly by enhancing vitamin uptake into skin.^{67,72} This combination inhibits tanning and immunosuppression in mice and tanning in human beings.

Vitamin A

The two main forms of vitamin A used in topical form are retinoids and carotenoids. The carotenoids on the skin scavenge ¹O₂ and quench lipid peroxidation.⁷³ Upon UV irradiation, the concentrations of human skin carotenoids, β -carotene and lycopene, are markedly reduced.⁷⁴ In the topical form, retinoids are commonly found in sunscreens and skin care cosmetics. The safety of retinyl palmitate, the storage form of vitamin A (retinol) has come under scrutiny because of animal studies suggesting it has photocarcinogenic effects upon UV irradiation. However, evidence from long-standing use of topical retinoids in clinical medicine demonstrates that they

are safe.⁷⁵ Retinol and its forms (tretinoin, isotretinoin, and tazarotene) are marketed as having anti-aging properties. The mechanism of action of these molecules is to bind to the nuclear receptors, retinoic acid receptors, and retinoid X, which will inhibit AP-1 and MMP-1 expression.⁵⁶ The benefits are increased collagen production and increasing epidermal thickness.

Selenium

Selenium is an essential element to optimize the activity of GSH peroxidase and thioredoxin reductase, and it also serves as a cofactor for vitamin E regeneration. In general, selenium sulfide and L-selenomethionine are the common forms used for topical delivery. The latter form has shown to have superior transepidermal delivery.⁷⁶ Topical L-selenomethionine increases the minimal erythema dose in human subjects.⁷⁷ When combined with vitamin E, selenium has shown to diminish UV-induced blistering, pigmentation, and skin tumors in mice studies.⁷⁸

Silymarin

Silymarin from the milk thistle plant contains a combination of 3 flavonoids, silybin, silydianin, and silychristin. Of these, silybin has the highest biologic potency to scavenge ROS and prevent lipoprotein oxidation. Topical application of silymarin inhibits sunburn cells, decreases pyrimidine dimers, and decreases skin tumors in hairless mice.^{79,80}

Tea polyphenols

Tea contains a rich level of polyphenols in the forms of epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate. Unfermented tea extract has a very high antioxidant activity, which diminishes in the making of commercial green, black, and oolong tea. Like other AOx, tea polyphenols are inherently unstable and a large portion of their biological activity is lost over a short duration. The topical formulation of polyphenol has been stabilized by butylated hydroxytoluene to reduce its susceptibility to oxidation.⁸¹ Hence, it is important to note that not all products containing tea extracts exhibit the same level of AOx properties. As AOx, tea polyphenols are more potent than vitamins C and E in scavenging ROS.⁸² In addition, tea polyphenols, specifically epigallocatechin-3-gallate, has anti-inflammatory and anticarcinogenic effects^{43,83} and can inhibit collagenase activity. In human studies, erythema and LC depletion have been examined.⁴⁴

Soy isoflavones

Soybeans contain isoflavones in the forms of genistein and daidzein. Diets high in soybeans are protective against various cancers and cardiovascular disease.⁸⁴ Isoflavones have been found to be anticarcinogenic through scavengers of peroxy and lipid radicals. Topical application of genistein has shown to decrease UV-induced oxidative damages, such as immunosuppression and inflammation.^{80,85,86}

PART III: ANTIOXIDANTS IN PHOTOPROTECTION

Sunscreen remains one of the most widely adopted strategies by the public to protect themselves from UVR. However, because of inadequate application and compensatory exposure where users of sunscreens tend to stay out in the sun longer, the degree of UV protection is much lower in practice than stated in the product labels. Furthermore, current sunscreens on the market tend to offer more UVB than UVA protection. Sunscreens may not offer adequate protection against UVA-induced ROS. In fact, Haywood et al⁸⁷ has shown that sunscreens with broad-spectrum UV protection only reduce free radical formation by 55%. Therefore, topical delivery of AOx can provide additional benefit to complement the protection from UV filters.

The protective benefit derived from combining AOx with sunscreen has been demonstrated in human studies. In a study by Matsui et al,⁸⁸ participants received two topical products: one sunscreen with an SPF 25 (SS) and the same sunscreen with an AOx mixture of caffeine, vitamin E and vitamin C, Echinacea pallida extract, gorgonian extract, and chamomile essential oil (SS+AOx). After UVR to the skin, the SS+AOx group had a 17% greater reduction in MMP-1 levels compared with the SS group. Both the SS and SS+AOx groups also protected against the depletion of LC. Wu et al⁴⁶ used a similar study design with an AOx preparation containing vitamin C, vitamin E, chamomile extract, Echinacea pallida extract, and caffeine. The investigators found the SS+AOx group had significant protection against MMP-9 induction, pigment formation, and markers associated with epidermal hyperproliferation, when compared with SS or AOx alone. These data add to the growing knowledge that AOx can add value to sunscreens but more *in vivo* research is needed to determine the best AOx to use in sunscreen formulations.

Despite the potential benefit, formulating products that combine AOx with sunscreen is a challenge. To ensure the efficacy of AOx in the final products, a number of technical requirements must

be fulfilled. First, AOxS need to have a high anti-oxidative capacity and be present in high concentration. Second, AOxS need to be stable in the final formulation. In general, AOxS are inherently unstable. In the case of vitamin E and C, tocopheryl acetate (a stabilized form of tocopherol) and ascorbyl palmitate (a stabilized form of ascorbic acid) are used as substitutes. However, these substitutes have very low biological activity. Other AOxS, such as ubiquinone, idebenone, and kinetin are degraded upon UV exposure.^{89,90} Third, AOxS need to penetrate the stratum corneum and maintain adequate concentrations in the epidermis and dermis. On the other hand, it is desirable to keep UV filters on the skin surface and not penetrate the skin. The conflicting goals for delivering AOxS and UV filters create additional challenges in the final formulation. Wang et al⁹¹ showed that sunscreens contained AOxS protected against free radicals, but nearly all of these tested sunscreen products had no or very minimal AOx capacity to neutralize the free radicals. In fact, the study showed that the radical protection is entirely from the UVA filters in the sunscreens. Many sunscreen products on the market claim to offer AOx protection, but they have inadequate or no AOx capacity to achieve any meaningful protection against free radicals.

CONCLUSION

ROS from endogenous and exogenous sources, such as UVR and pollution, can damage the DNA, lipid membrane, and protein structures, and also play a role in the acceleration of photoaging and the development of skin cancer. Although the body's innate AOx defense can neutralize ROS, these protective agents may be overwhelmed and depleted when faced with an excessive amount of oxidative stress. Delivery of topical AOxS has the potential to provide additional benefits, but there remain many challenges in effectively incorporating AOxS in skin care and sunscreen formulations.

REFERENCES

- Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. New York: Oxford University Press; 2007.
- Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 2000;29:222-30.
- Turrens JF. Superoxide production by the mitochondrial respiratory chain. *Biosci Rep* 1997;17:3-8.
- Dalton TP, Shertzer HG, Puga A. Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* 1999;39:67-101.
- Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch Biochem Biophys* 1993;300:535-43.
- Sies H. Strategies of antioxidant defense. *Eur J Biochem* 1993;215:213-9.
- Masaki H, Atsumi T, Sakurai H. Detection of hydrogen peroxide and hydroxyl radicals in murine skin fibroblasts under UVB irradiation. *Biochem Biophys Res Commun* 1995;206:474-9.
- Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 2002;138:1462-70.
- Heck DE, Vetrano AM, Mariano TM, Laskin JD. UVB light stimulates production of reactive oxygen species: unexpected role for catalase. *J Biol Chem* 2003;278:22432-6.
- Cadet J, Douki T, Ravanat JL, Di Mascio P. Sensitized formation of oxidatively generated damage to cellular DNA by UVA radiation. *Photochem Photobiol Sci* 2009;8:903-11.
- Ananthaswamy HN, Pierceall WE. Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem Photobiol* 1990;52:1119-36.
- Yu H, Xia Q, Yan J, Herreno-Saenz D, Wu YS, Tang IW, et al. Photoirradiation of polycyclic aromatic hydrocarbons with UVA light—a pathway leading to the generation of reactive oxygen species, lipid peroxidation, and DNA damage. *Int J Environ Res Public Health* 2006;3:348-54.
- Liu ZS, Lu YH, Rosenstein B, Lebwohl M, Wei HC. Benzo a pyrene enhances the formation of 8-hydroxy-2'-deoxyguanosine by ultraviolet A radiation in calf thymus DNA and human epidermoid carcinoma cells. *Biochemistry* 1998;37:10307-12.
- Saladi R, Austin L, Gao DY, Lu YH, Phelps R, Lebwohl M, et al. The combination of benzo a pyrene and ultraviolet A causes an in vivo time-related accumulation of DNA damage in mouse skin. *Photochem Photobiol* 2003;77:413-9.
- Shyong EQ, Lu YH, Goldstein A, Lebwohl M, Wei HC. Synergistic enhancement of H₂O₂ production in human epidermoid carcinoma cells by benzo a pyrene and ultraviolet A radiation. *Toxicol Appl Pharmacol* 2003;188:104-9.
- Wang Y, Saladi R, Wei H. Synergistic carcinogenesis of chemical carcinogens and long wave-length UVA radiation. *Trends Photochem Photobiol* 2003;10:31-45.
- Cadet J, Douki T. Oxidatively generated damage to DNA by UVA radiation in cells and human skin. *J Invest Dermatol* 2011;131:1005-7.
- Kielbassa C, Roza L, Epe B. Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis* 1997;18:811-6.
- Beehler BC, Przybyszewski J, Box HB, Kulesz-Martin MF. Formation of 8-hydroxydeoxyguanosine within DNA of mouse keratinocytes exposed in culture to UVB and H₂O₂. *Carcinogenesis* 1992;13:2003-7.
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006;160:1-40.
- Nishigori C, Hattori Y, Toyokuni S. Role of reactive oxygen species in skin carcinogenesis. *Antioxid Redox Signal* 2004;6:561-70.
- Birch-Machin MA, Tindall M, Turner R, Haldane F, Rees JL. Mitochondrial DNA deletions in human skin reflect photo-rather than chronologic aging. *J Invest Dermatol* 1998;110:149-52.
- Berneburg M, Grether-Beck S, Kurten V, Ruzicka T, Briviba K, Sies H, et al. Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion. *J Biol Chem* 1999;274:15345-9.
- Sander CS, Chang H, Salzmann S, Muller CS, Ekanayake-Mudiyanselage S, Elsner P, et al. Photoaging is associated with

- protein oxidation in human skin in vivo. *J Invest Dermatol* 2002;118:618-25.
25. Godar DE. UVA1 radiation triggers two different final apoptotic pathways. *J Invest Dermatol* 1999;112:3-12.
 26. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;11:298-300.
 27. Wlaschek M, Briviba K, Stricklin GP, Sies H, Scharffetter-Kochanek K. Singlet oxygen may mediate the ultraviolet A-induced synthesis of interstitial collagenase. *J Invest Dermatol* 1995;104:194-8.
 28. Sardy M. Role of matrix metalloproteinases in skin aging. *Connect Tissue Res* 2009;50:132-8.
 29. Schroeder P, Gremmel T, Berneburg M, Krutmann J. Partial depletion of mitochondrial DNA from human skin fibroblasts induces a gene expression profile reminiscent of photoaged skin. *J Invest Dermatol* 2008;128:2297-303.
 30. Koch H, Wittern KP, Bergemann J. In human keratinocytes the common deletion reflects donor variabilities rather than chronologic aging and can be induced by ultraviolet A irradiation. *J Invest Dermatol* 2001;117:892-7.
 31. Kawaguchi Y, Tanaka H, Okada T, Konishi H, Takahashi M, Ito M, et al. Effect of reactive oxygen species on the elastin mRNA expression in cultured human dermal fibroblasts. *Free Radic Biol Med* 1997;23:162-5.
 32. Bernstein EF. Reactive oxygen species activate the human elastin promoter in a transgenic model of cutaneous photoaging. *Dermatol Surg* 2002;28:132-5.
 33. Uitto J. The role of elastin and collagen in cutaneous aging: intrinsic aging versus photoexposure. *J Drugs Dermatol* 2008;7:512-6.
 34. Halliday GM. Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis. *Mutat Res* 2005;571:107-20.
 35. Ullrich SE. Mechanisms underlying UV-induced immune suppression. *Mutat Res* 2005;571:185-205.
 36. Shreedhar V, Giese T, Sung VW, Ullrich SE. A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. *J Immunol* 1998;160:3783-9.
 37. Bestak R, Halliday GM. Chronic low-dose UVA irradiation induces local suppression of contact hypersensitivity, Langerhans cell depletion and suppressor cell activation in C3H/HeJ mice. *Photochem Photobiol* 1996;64:969-74.
 38. Stoebner PE, Poosti R, Djoukelfit K, Martinez J, Meunier L. Decreased human epidermal antigen-presenting cell activity after ultraviolet A exposure: dose-response effects and protection by sunscreens. *Br J Dermatol* 2007;156:1315-20.
 39. Moyal DD, Fourtanier AM. Broad-spectrum sunscreens provide better protection from solar ultraviolet-simulated radiation and natural sunlight-induced immunosuppression in human beings. *J Am Acad Dermatol* 2008;58(Suppl):S149-54.
 40. Baron ED, Fourtanier A, Compan D, Medaisko C, Cooper KD, Stevens SR. High ultraviolet A protection affords greater immune protection confirming that ultraviolet A contributes to photoimmunosuppression in humans. *J Invest Dermatol* 2003;121:869-75.
 41. Wolf P, Hoffmann C, Quehenberger F, Grinschgl S, Kerl H. Immune protection factors of chemical sunscreens measured in the local contact hypersensitivity model in humans. *J Invest Dermatol* 2003;121:1080-7.
 42. Halliday GM, Russo PA, Yuen KS, Robertson BO. Effect of inhibitors of oxygen radical and nitric oxide formation on UV radiation-induced erythema, immunosuppression and carcinogenesis. *Redox Rep* 1999;4:316-8.
 43. Katiyar SK, Matsui MS, Elmetts CA, Mukhtar H. Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. *Photochem Photobiol* 1999;69:148-53.
 44. Elmetts CA, Singh D, Tubesing K, Matsui M, Katiyar S, Mukhtar H. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J Am Acad Dermatol* 2001;44:425-32.
 45. Camouse MM, Domingo DS, Swain FR, Conrad EP, Matsui MS, Maes D, et al. Topical application of green and white tea extracts provides protection from solar-simulated ultraviolet light in human skin. *Exp Dermatol* 2009;18:522-6.
 46. Wu Y, Matsui MS, Chen JZ, Jin X, Shu CM, Jin GY, et al. Antioxidants add protection to a broad-spectrum sunscreen. *Clin Exp Dermatol* 2011;36:178-87.
 47. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;39:44-84.
 48. Gensler HL, Magdaleno M. Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet irradiation. *Nutr Cancer* 1991;15:97-106.
 49. Burke KE, Clive J, Combs GF Jr, Commisso J, Keen CL, Nakamura RM. Effects of topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *Nutr Cancer* 2000;38:87-97.
 50. Tian FF, Zhang FF, Lai XD, Wang LJ, Yang L, Wang X, et al. Nrf2-mediated protection against UVA radiation in human skin keratinocytes. *Biosci Trends* 2011;5:23-9.
 51. Hirota A, Kawachi Y, Itoh K, Nakamura Y, Xu X, Banno T, et al. Ultraviolet A irradiation induces NF-E2-related factor 2 activation in dermal fibroblasts: protective role in UVA-induced apoptosis. *J Invest Dermatol* 2005;124:825-32.
 52. Shindo Y, Witt E, Packer L. Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J Invest Dermatol* 1993;100:260-5.
 53. Shindo Y, Witt E, Han D, Packer L. Dose-response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. *J Invest Dermatol* 1994;102:470-5.
 54. Thiele JJ, Traber MG, Packer L. Depletion of human stratum corneum vitamin E: an early and sensitive in vivo marker of UV induced photo-oxidation. *J Invest Dermatol* 1998;110:756-61.
 55. Rhie G, Shin MH, Seo JY, Choi WW, Cho KH, Kim KH, et al. Aging- and photoaging-dependent changes of enzymic and nonenzymic antioxidants in the epidermis and dermis of human skin in vivo. *J Invest Dermatol* 2001;117:1212-7.
 56. Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, et al. Molecular basis of sun-induced premature skin aging and retinoid antagonism. *Nature* 1996;379:335-9.
 57. Campos PM, Goncalves GM, Gaspar LR. In vitro antioxidant activity and in vivo efficacy of topical formulations containing vitamin C and its derivatives studied by non-invasive methods. *Skin Res Technol* 2008;14:376-80.
 58. Varani J, Spearman D, Perone P, Fligiel SE, Datta SC, Wang ZQ, et al. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. *Am J Pathol* 2001;158:931-42.
 59. Darr D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 1992;127:247-53.
 60. Nakamura T, Pinnell SR, Darr D, Kurimoto I, Itami S, Yoshikawa K, et al. Vitamin C abrogates the deleterious effects of UVB radiation on cutaneous immunity by a

- mechanism that does not depend on TNF- α . *J Invest Dermatol* 1997;109:20-4.
61. Nayama S, Takehana M, Kanke M, Itoh S, Ogata E, Kobayashi S. Protective effects of sodium-L-ascorbyl-2 phosphate on the development of UVB-induced damage in cultured mouse skin. *Biol Pharm Bull* 1999;22:1301-5.
 62. Pinnell SR, Yang H, Omar M, Monteiro-Riviere N, DeBuys HV, Walker LC, et al. Topical L-ascorbic acid: percutaneous absorption studies. *Dermatol Surg* 2001;27:137-42.
 63. Lopez-Torres M, Thiele JJ, Shindo Y, Han D, Packer L. Topical application of alpha-tocopherol modulates the antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. *Br J Dermatol* 1998;138:207-15.
 64. Bissett DL, Chatterjee R, Hannon DP. Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol Photoimmunol Photomed* 1990;7:56-62.
 65. Jurkiewicz BA, Bissett DL, Buettner GR. Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. *J Invest Dermatol* 1995;104:484-8.
 66. Steenvoorden DP, Beijersbergen van Henegouwen G. Protection against UV-induced systemic immunosuppression in mice by a single topical application of the antioxidant vitamins C and E. *Int J Radiat Biol* 1999;75:747-55.
 67. Oresajo C, Stephens T, Hino PD, Law RM, Yatskayer M, Foltis P, et al. Protective effects of a topical antioxidant mixture containing vitamin C, ferulic acid, and phloretin against ultraviolet-induced photodamage in human skin. *J Cosmet Dermatol* 2008;7:290-7.
 68. Ricciarelli R, Maroni P, Ozer N, Zingg JM, Azzi A. Age-dependent increase of collagenase expression can be reduced by alpha-tocopherol via protein kinase C inhibition. *Free Radic Biol Med* 1999;27:729-37.
 69. Chen W, Barthelman M, Martinez J, Alberts D, Gensler HL. Inhibition of cyclobutane pyrimidine dimer formation in epidermal p53 gene of UV-irradiated mice by alpha-tocopherol. *Nutr Cancer* 1997;29:205-11.
 70. McVean M, Liebler DC. Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. *Mol Carcinog* 1999;24:169-76.
 71. Lin JY, Selim MA, Shea CR, Grichnik JM, Omar MM, Monteiro-Riviere NA, et al. UV photoprotection by combination topical antioxidants vitamin C and vitamin E. *J Am Acad Dermatol* 2003;48:866-74.
 72. Lin FH, Lin JY, Gupta RD, Tournas JA, Burch JA, Selim MA, et al. Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. *J Invest Dermatol* 2005;125:826-32.
 73. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr* 1995;62:1315S-21S.
 74. Ribaya-Mercado JD, Garmyn M, Gilchrist BA, Russell RM. Skin lycopene is destroyed preferentially over beta-carotene during ultraviolet irradiation in humans. *J Nutr* 1995;125:1854-9.
 75. Wang SQ, Dusza SW, Lim HW. Safety of retinyl palmitate in sunscreens: a critical analysis. *J Am Acad Dermatol* 2010;63:903-6.
 76. Lin CH, Fang CL, Al-Suwayeh SA, Yang SY, Fang JY. In vitro and in vivo percutaneous absorption of seleno-L-methionine, an antioxidant agent, and other selenium species. *Acta Pharmacol Sin* 2011;32:1181-90.
 77. Burke KE, Burford RG, Combs GF Jr, French IW, Skeffington DR. The effect of topical L-selenomethionine on minimal erythema dose of ultraviolet irradiation in humans. *Photodermatol Photoimmunol Photomed* 1992;9:52-7.
 78. Burke KE, Clive J, Combs GF Jr, Nakamura RM. Effects of topical L-selenomethionine with topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *J Am Acad Dermatol* 2003;49:458-72.
 79. Afaq F, Adhami VM, Ahmad N, Mukhtar H. Botanical antioxidants for chemoprevention of photocarcinogenesis. *Front Biosci* 2002;7:d784-92.
 80. Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. *J Natl Cancer Inst* 1997;89:556-66.
 81. Dvorakova K, Dorr RT, Valcic S, Timmermann B, Alberts DS. Pharmacokinetics of the green tea derivative, EGCG, by the topical route of administration in mouse and human skin. *Cancer Chemother Pharmacol* 1999;43:331-5.
 82. Rice-Evans C. Implications of the mechanisms of action of tea polyphenols as antioxidants in vitro for chemoprevention in humans. *Proc Soc Exp Biol Med* 1999;220:262-6.
 83. Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS, et al. Topical applications of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. *Proc Natl Acad Sci U S A* 2002;99:12455-60.
 84. Wei H, Saladi R, Lu Y, Wang Y, Palep SR, Moore J, et al. Isoflavone genistein: photoprotection and clinical implications in dermatology. *J Nutr* 2003;133:3811S-9S.
 85. Moore JO, Wang Y, Stebbins WG, Gao D, Zhou X, Phelps R, et al. Photoprotective effect of isoflavone genistein on ultraviolet B-induced pyrimidine dimer formation and PCNA expression in human reconstituted skin and its implications in dermatology and prevention of cutaneous carcinogenesis. *Carcinogenesis* 2006;27:1627-35.
 86. Svobodova A, Psotova J, Walterova D. Natural phenolics in the prevention of UV-induced skin damage: a review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2003;147:137-45.
 87. Haywood R, Wardman P, Sanders R, Linge C. Sunscreens inadequately protect against ultraviolet-A-induced free radicals in skin: implications for skin aging and melanoma? *J Invest Dermatol* 2003;121:862-8.
 88. Matsui MS, Hsia A, Miller JD, Hanneman K, Scull H, Cooper KD, et al. Non-sunscreen photoprotection: antioxidants add value to a sunscreen. *J Invest Dermatol Symp Proc* 2009;14:56-9.
 89. Lin JY, Lin FH, Burch JA, Selim MA, Monteiro-Riviere NA, Grichnik JM, et al. Alpha-lipoic acid is ineffective as a topical antioxidant for photoprotection of skin. *J Invest Dermatol* 2004;123:996-8.
 90. Tournas JA, Lin FH, Burch JA, Selim MA, Monteiro-Riviere NA, Zielinski JE, et al. Ubiquinone, idebenone, and kinetin provide ineffective photoprotection to skin when compared to a topical antioxidant combination of vitamins C and E with ferulic acid. *J Invest Dermatol* 2006;126:1185-7.
 91. Wang SQ, Stanfield JW, Osterwalder U. In vitro assessments of UVA protection by popular sunscreens available in the United States. *J Am Acad Dermatol* 2008;59:934-42.
 92. Traikovich SS. Use of topical ascorbic acid and its effects on photodamaged skin topography. *Arch Otolaryngol Head Neck Surg* 1999;125:1091-8.
 93. Potapenko A, Abijev GA, Pistsov M, Roshchupkin DI, Vladimirov Y, Pliquett F, et al. PUVA-induced erythema and changes in mechano-electrical properties of skin: inhibition by tocopherols. *Arch Dermatol Res* 1984;276:12-6.
 94. Dreher F, Denig N, Gabard B, Schwindt DA, Maibach HI. Effect of topical antioxidants on UV-induced erythema formation

- when administered after exposure. *Dermatology* 1999;198:52-5.
95. Olsen EA, Katz HI, Levine N, Nigra TP, Pochi PE, Savin RC, et al. Tretinoin emollient cream for photodamaged skin: results of 48-week, multicenter, double-blind studies. *J Am Acad Dermatol* 1997;37:217-26.
 96. Burke KE, Combs GF Jr, Gross EG, Bhuyan KC, Abu-Libdeh H. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. *Nutr Cancer* 1992;17:123-37.
 97. Lahiri-Chatterjee M, Katiyar SK, Mohan RR, Agarwal R. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. *Cancer Res* 1999;59:622-32.
 98. Katiyar SK. Treatment of silymarin, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin. *Int J Oncol* 2002;21:1213-22.
 99. Kim J, Hwang JS, Cho YK, Han Y, Jeon YJ, Yang KH. Protective effects of (-)-epigallocatechin-3-gallate on UVA- and UVB-induced skin damage. *Skin Pharmacol Appl Skin Physiol* 2001;14:11-9.
 100. Lin JY, Tournas JA, Burch JA, Monteiro-Riviere NA, Zielinski J. Topical isoflavones provide effective photoprotection to skin. *Photodermatol Photoimmunol Photomed* 2008;24:61-6.
 101. Widyarini S, Spinks N, Husband AJ, Reeve VE. Isoflavonoid compounds from red clover (*Trifolium pratense*) protect from inflammation and immune suppression induced by UV radiation. *Photochem Photobiol* 2001;74:465-70.
 102. Reeve VE, Widyarini S, Domanski D, Chew E, Barnes K. Protection against photoaging in the hairless mouse by the isoflavone equol. *Photochem Photobiol* 2005;81:1548-53.
 103. Widyarini S, Husband AJ, Reeve VE. Protective effect of the isoflavonoid equol against hairless mouse skin carcinogenesis induced by UV radiation alone or with a chemical cocarcinogen. *Photochem Photobiol* 2005;81:32-7.
 104. Saija A, Tomaino A, Trombetta D, De Pasquale A, Uccella N, Barbuzzi T, et al. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. *Int J Pharm* 2000;199:39-47.
 105. Staniforth V, Chiu LT, Yang NS. Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse. *Carcinogenesis* 2006;27:1803-11.
 106. Hwang YP, Oh KN, Yun HJ, Jeong HG. The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. *J Dermatol Sci* 2011;61:23-31.
 107. Birt DF, Mitchell D, Gold B, Pour P, Pinch HC. Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. *Anticancer Res* 1997;17:85-91.
 108. Gonzalez S, Pathak MA, Cuevas J, Villarrubia VG, Fitzpatrick TB. Topical or oral administration with an extract of *Polypodium leucotomos* prevents acute sunburn and psoralen-induced phototoxic reactions as well as depletion of Langerhans cells in human skin. *Photodermatol Photoimmunol Photomed* 1997;13:50-60.
 109. Alcaraz MV, Pathak MA, Rius F, Kollias N, Gonzalez S. An extract of *Polypodium leucotomos* appears to minimize certain photoaging changes in a hairless albino mouse animal model: a pilot study. *Photodermatol Photoimmunol Photomed* 1999;15:120-6.
 110. Philips N, Smith J, Keller T, Gonzalez S. Predominant effects of *Polypodium leucotomos* on membrane integrity, lipid peroxidation, and expression of elastin and matrixmetalloproteinase-1 in ultraviolet radiation exposed fibroblasts, and keratinocytes. *J Dermatol Sci* 2003;32:1-9.
 111. Sime S, Reeve VE. Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical Pycnogenol. *Photochem Photobiol* 2004;79:193-8.
 112. Afaq F, Adhami VM, Ahmad N. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. *Toxicol Appl Pharmacol* 2003;186:28-37.
 113. Aziz MH, Reagan-Shaw S, Wu J, Longley BJ, Ahmad N. Chemoprevention of skin cancer by grape constituent resveratrol: relevance to human disease? *FASEB J* 2005;19:1193-5.
 114. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol* 2003;552:335-44.