REVIEW

The role of antioxidants in photoprotection: A critical review

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

Abbreviations used:

AOx:	antioxidant
AP-1:	activation protein-1
ATP:	adenosine triphosphate
GSH:	glutathione
H_2O_2:	hydrogen peroxide
LC:	Langerhans cell
MMP:	matrix metalloproteinase
NF-κB:	nuclear factor-κB
$O_2^{-\bullet}$:	superoxide anion
$OH^{\bullet}$:	hydroxyl radical
ROS:	reactive oxygen species
SOD:	superoxide dismutase
UV:	ultraviolet
UVR:	ultraviolet radiation
$^1O_2$:	singlet oxygen

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.

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The most common oxygen-based ROS are: superoxide anion ($O_2^{-}\bullet$), peroxide, hydroxyl radical (OH•), 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•, one of the most unstable ROS that exists in a biological system.[5] The half-life of OH• 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• 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, benzoapyrene and its intermediates, act as photosensitizers, which upon UVA exposure, synergistically increase production of superoxide and $^1O_2$.[12-16]

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.

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• that abstracts a hydrogen atom from nearby unsaturated fatty acid. This forms lipid molecules with extra electrons, which form peroxyl 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

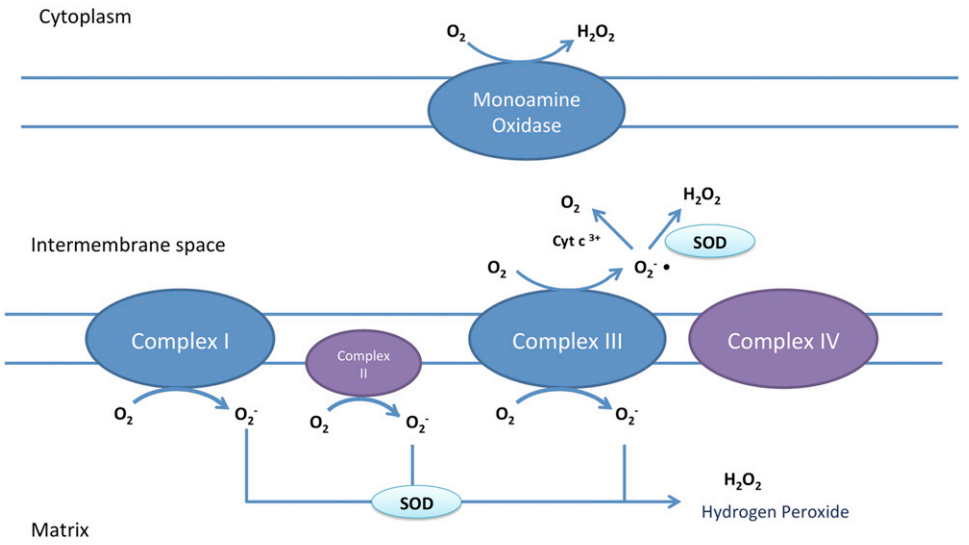

Fig 1. Formation of superoxide in mitochondrial respiratory chain. Complexes in mitochondrial respiratory chain leak electrons to oxygen-producing superoxide anion (O_2^-•). Increased concentrations of O_2^-• may reduce transition metals, which in turn react with hydrogen peroxide (H_2O_2)-producing hydroxyl radicals (OH•) or may react with nitric oxide to form peroxynitrite. Both OH• and peroxynitrite are strong oxidants that indiscriminately react with DNA, lipids, and proteins. O_2^-• 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

Harman[26] 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-B (NF-κB) 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$-\beta$ 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-kB 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 al[42] used AOxs to evaluate UVA-induced immunosuppression. In the presence of topical L-NMMA (nitric oxide inhibitor), iron chelator 2,2'-dipyridl, and the SOD-mimicking agent 4-hydroxy-tempol, 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

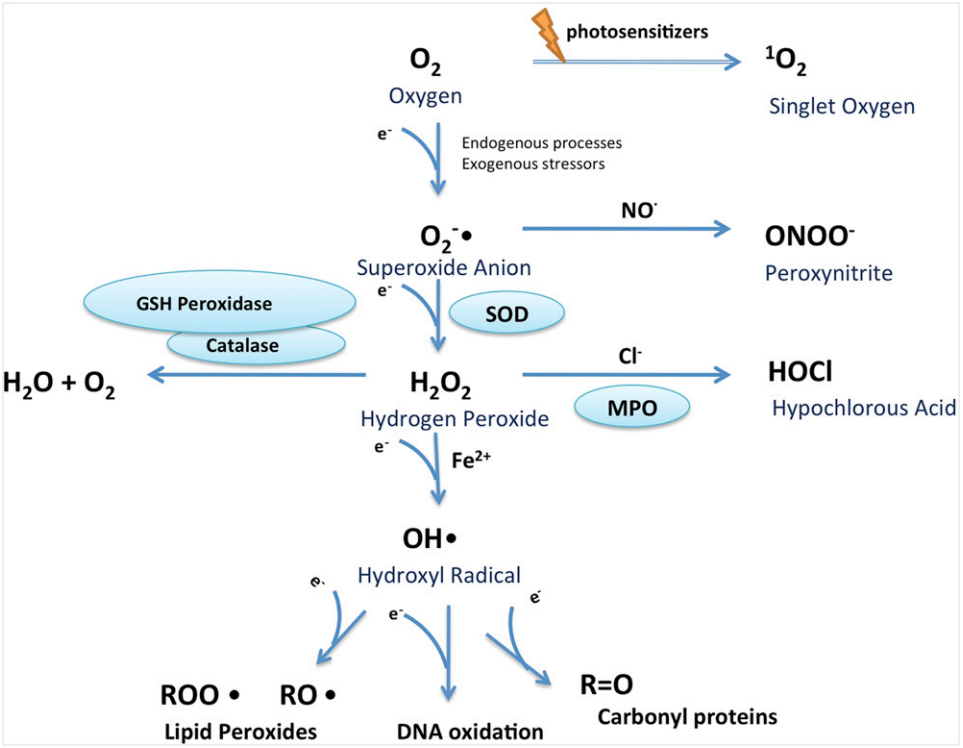

Fig 2. Generation of reactive oxygen species (ROS). Oxygen molecule can be converted into singlet oxygen ($^1O_2$) or superoxide anion (O_2^-•). O_2^-• is extremely unstable and can be further converted to hydrogen peroxide (H_2O_2) either spontaneously or enzymatically by superoxide dismutase (*SOD*). H_2O_2 is more stable than O_2^-• 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•) in presence of iron (Fe^{2+}) via Fenton reaction (ie, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH$• + hydroxyl ion). OH• 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-kB pathways have been implicated in cell proliferation and apoptosis leading to carcinogenesis. Halliday[34] 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

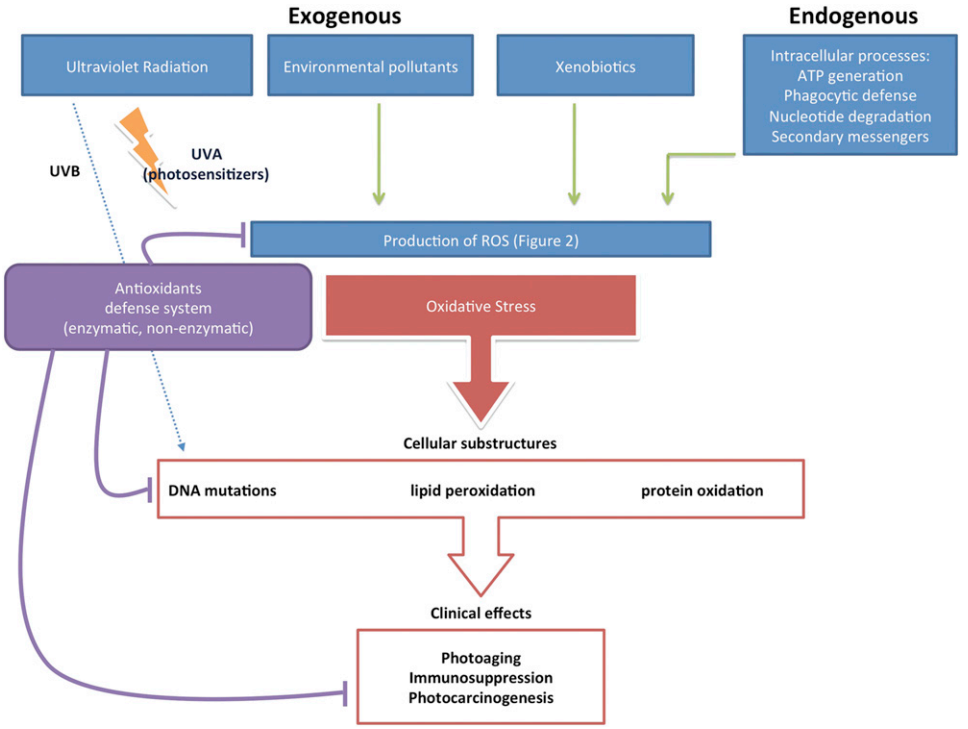

Fig 3. Cellular and clinical effects of reactive oxygen species (*ROS*). ROS are generated from exogenous and endogenous sources. On cellular level, ROS has potential to cause DNA mutation, lipid peroxidation, and protein oxidation. On clinical level, ROS plays a role in photoaging, immunosuppression, 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 suberythmogenic 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]

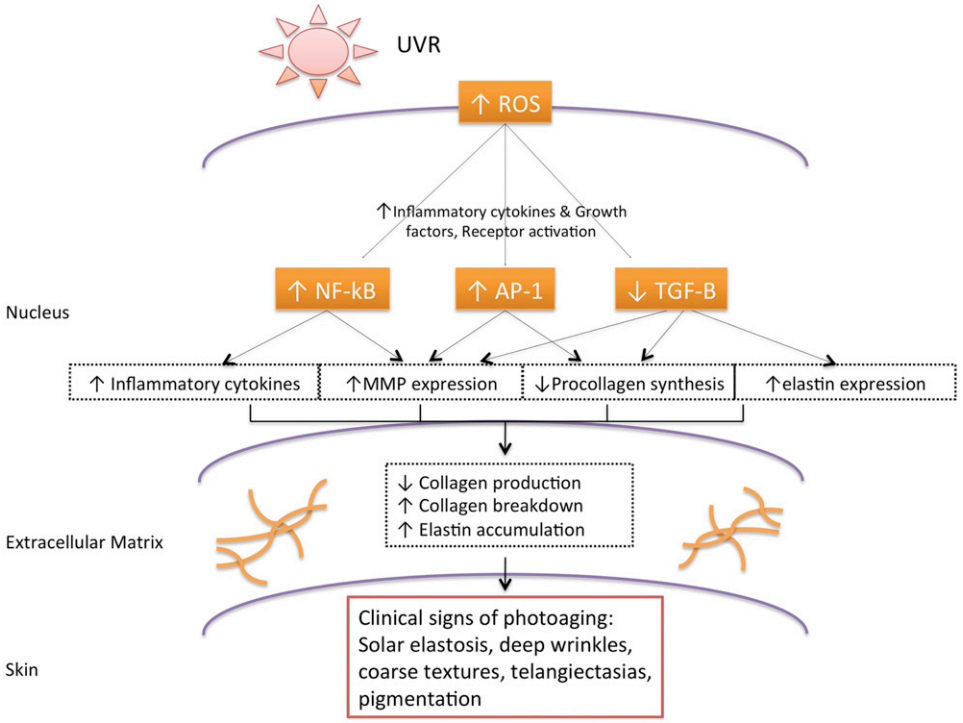

Fig 4. Role of reactive oxygen species (*ROS*) in photoaging. ROS from exogenous (eg, ultraviolet [*UV*] radiation) and endogenous sources initiates signal transduction cascade resulting in up-regulation of AP-1, NF-kB, and down-regulation of transforming growth factor (*TGF*)-β. Downstream, NF-kb signals increase in interleukin-1 and tumor necrosis factor-alfa 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, epicathechin-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 $^1O_2$ 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 erythemal 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 antioxidative activity, which diminishes in the making of commercial green, black, and oolong tea. Like other AOxs, 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 AOxs, 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 peroxyl 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 al[87] 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 AOxs 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 al[46] 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 AOxs can add value to sunscreens but more in vivo research is needed to determine the best AOxs to use in sunscreen formulations.

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

be fulfilled. First, AOxs need to have a high antioxidative 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 al[91] 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.

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