Role of antioxidants in the skin: Anti-aging effects

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Introduction

This paper explains the role antioxidants play in skin aging, as well as details several different antioxidants

Conclusions

Much of the damage induced on skin is through ultraviolent (UV) light, which generates reactive oxygen species (ROS). These ROS devolve into different types of ROS that affect proteins and lipids within cells, throwing off signaling within the skin cells (keratinocytes). Antioxidants neutralize these ROS by donating electrons without becoming ROS, themselves.

Different types of ROS can impact the skin cells differently - some will darken skin, while others will lighten it, depending on their impact on melanocytes (skin darkening cells).

UV also increases collagen breakdown and decreases its synthesis.

Amendments

This review was written by a person who is working at a cosmetics company - there may be a conflict of interest there; however, the review did go through the peer review process and is published in a reputable scientific journal. Also, note that this reviewprovides no primary data - only references to other publications.

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Invited review article

Role of antioxidants in the skin: Anti-aging effects

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ABSTRACT

Intracellular and extracellular oxidative stress initiated by reactive oxygen species (ROS) advance skin aging, which is characterized by wrinkles and atypical pigmentation. Because UV enhances ROS generation in cells, skin aging is usually discussed in relation to UV exposure. The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. In this review, the mechanisms of ROS generation and ROS elimination in the body are summarized. The effects of ROS generated in the skin and the roles of ROS in altering the skin are also discussed. In addition, the effects of representative antioxidants on the skin are summarized with a focus on skin aging.

Contents

1. Definition of ROS and the oxidation of biomolecules by ROS

ROS can be divided into two categories: oxygen molecules that have an unpaired electron and oxygen molecules that are in an excited state (Fig. 1). The former type includes superoxide anion radicals ($^{\cdot}O_2^-$), hydroxyl radicals ($^{\cdot}OH$), lipid peroxyl radicals ($LOO^{\cdot}$), and nitric oxide radicals ($NO^{\cdot}$). The latter type is singlet oxygen ($^1O_2$). Basically, $^{\cdot}O2^-$ are generated by some enzymatic reactions such as NADPH oxidase and xanthine oxidase, and as a byproduct of the respiratory chain reaction in mitochondria [1–3]. $NO^{\cdot}$ are also generated by nitric oxide synthase (NOS) [4].

The oxidative pathway of lipids and proteins is summarized in Fig. 1. $^{\cdot}O_2^-$ are generated first, and are spontaneously converted to hydrogen peroxide (H_2O_2) or are metabolized by superoxide dismutase (SOD). H_2O_2, which is more stable and plasma membrane permeable, yields $^{\cdot}OH$ in the presence of Fe^{2+} or Cu^+ through the Fenton reaction. $^{\cdot}OH$ and $^1O_2$ oxidize the unsaturated bonds of lipids to yield lipid peroxides and aldehydes such as

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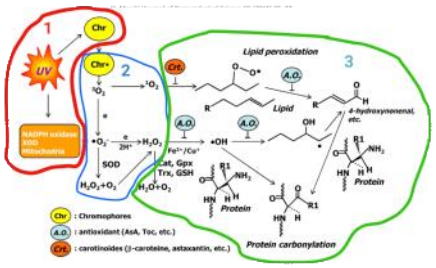
Reactive Oxygen Species (ROS) are split into two groups: 1. Oxygen missing an electron (so it searches for another electron to take away for itself) and 2. Oxygen with the proper electron number, but they are misplaced.



4-hydroxynonenal [5]. $^{\bullet}OH$ and the resulting aldehydes react with amino acid residues in proteins to produce carbonyl proteins.

2. Endogenous and exogenous antioxidants

ROS cause mutations in various species depending on the environment. Several ROS elimination systems have developed in mammalian tissues to eliminate ROS and protect cells. SOD catalyzes the dismutation of $^{\bullet}O_2^-$ into O_2 (oxygen molecule) and H_2O_2 [6], and catalase breaks down H_2O_2 into O_2 and H_2O [7]. The combination of SOD and catalase completely scavenges $^{\bullet}O_2^-$ initiated ROS. In addition to catalase, glutathione peroxidase (GPx) also breaks down H_2O_2 c in the presence of the reduced form of glutathione (GSH). GPx also decomposes lipid hydroperoxides into their corresponding alcohols [8]. Thioredoxin, a ubiquitous oxidoreductase enzyme, breaks down H_2O_2 in a NADPH-dependent reaction within cells [9]. Metallothionine, a heavy metal ion-induced cysteine-rich peptide, also functions as a ROS scavenger [10].

In response to excess oxidative stress, the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway functions to reinforce the intracellular antioxidant capacity. Nrf2, which is activated by the dissociation of Keap1, binds to an antioxidant response element and upregulates the transcription of several different types of genes [11]. The Nrf2 downstream genes identified to date can be categorized into several groups, including (i) intracellular redox-balancing proteins, such as γ-glutamylcysteine synthetase (a rate limiting enzyme of GSH synthesis), GPx, thioredoxin, thioredoxin reductase, peroxiredoxin, and heme oxygenase-1, (ii) phase II detoxifying enzymes, such as glutathione S transferase, NAD(P)H quinone oxidoreductase-1, and UDP-glucuronosyltransferase, and (iii) transporters, such as multidrug resistance-associated protein [12–20].

3. Generation of ROS in the skin

UV radiation is a potent initiator of ROS generation in the skin. The type(s) of ROS generated, however, depends on the UV wavelength. UVB mainly stimulates the production of $^{\bullet}O_2^-$ through the activation of NADPH oxidase and respiratory chain reactions [21,22], while UVA produces $^1O_2$ through a photosensitizing reaction with internal chromophores such as riboflavin and porphyrin. UVA also generates $^{\bullet}O_2^-$ through NADPH oxidase activation [23] and photosensitization of advanced glycation products [24].

The major type of ROS produced on the skin surface is $^1O_2$, which is generated by a photosensitizing reaction with UVA and porphyrins from bacterial flora living in the skin [25]. $^1O_2$ is oxidized to squalene, cholesterol, and to unsaturated acyl residues in the sebum to yield lipid hydroperoxides.

4. Role of oxidative stress/ROS in the skin

4.1. Inflammation

UVB radiation induces erythema in the skin, which is called a sunburn. UVB-induced erythema is attenuated by the NOS inhibitor NG-monomethyl-L-arginine and the cyclooxygenase (COX) inhibitor indomethacin [26]. ROS, including NO, induce skin erythema through prostaglandin E2 synthesis [27]. Expression of COX-2, a pivotal enzyme in prostaglandin E2 synthesis, is upregulated by ROS to stimulate the inflammation process [28].

4.2. Oxidation at the skin surface

Oxidized lipids and proteins induces alterations in skin conditions. Topical application of oxidized squalene (squalene monohydroperoxide) on the skin disrupts the skin barrier function as an acute response and induces skin roughness as a chronic response [29]. Alkyl aldehydes further oxidize lipid hydroperoxides and proteins to produce carbonylated proteins in the stratum corneum (SCCP). The SCCP levels increase following UV-exposure [30] and during the winter season [31]. In addition, patients suffering from atopic dermatitis have higher levels of SCCP compared with normal subjects [32]. SCCP levels appear to reflect the degree of oxidative stress in the skin induced by the environment. Thus, oxidative stress initiated by ROS alters skin conditions.

4.3. Sebaceous glands

UV radiation-induced oxidative stress stimulates sebaceous gland function, eventually increasing sebum secretion due to increased levels of oxidized lipids, triglyceride hydroperoxides, and cholesterol hydroperoxides [33]. In the inflammatory process of acne vulgaris, *Propionibacterium acnes* (*P. acnes*), a Gram-positive anaerobic bacterium, produces coproporphyrin, which generates $^1O_2$ during UVA exposure, and therefore has a critical role in the development of the inflammatory lesions of acne. The inflammatory reaction is further stimulated by $^{\bullet}O_2^-$-generated from keratinocytes infected with *P. acnes* [34].

4.4. Melanogenesis

ROS has a paradoxical action on melanocytes because it not only enhances depigmentation, but also increases pigmentation in the skin. An example of melanocyte degeneration induced by oxidative stress is vitiligo, characterized by circumscribed depigmented macules in the skin [35]. The skin of patients with vitiligo vulgaris contains high levels of SOD and low levels of catalase [36]. An imbalance of the ROS scavenging system results in the accumulation of H_2O_2 in the skin. Keratinocytes are a source of the H_2O_2 affecting melanocytes [37]. H_2O_2 readily crosses the cell membrane and is therefore easily transferred to melanocytes from the keratinocytes. The transfer of H_2O_2 is thought to be one of the pathogenetic mechanisms of vitiligo.

ROS can also accelerate skin pigmentation. Keratinocytes adjacent to melanocytes intensively contribute to UV-induced skin pigmentation. Among ROS, NO˙ derived from keratinocytes acts to induce melanogenesis by increasing the amount of the melanogenic factors tyrosinase and tyrosinase-related protein 1 [38,39].

The contribution of ROS to melanogenesis has been demonstrated by studies using antioxidants. α-Melanocyte stimulating hormone, which is increased by UVB, is abolished by the addition of N-acetyl cysteine, a precursor of GSH [40]. In addition, stimulation by an endogenous antioxidant, metallothionein, also suppresses melanogenesis in melanocytes [41].

Furthermore, H_2O_2 activates epidermal phenylalanine hydroxylase (PAH), which is an enzyme that produces L-tyrosine from the essential amino acid L-phenylalanine, and thus contributes to melanogenesis by increasing the pool of L-tyrosine, the initial substrate of tyrosinase. In fact, PAH activity positively correlates with skin phototypes (I–VI) and exposure to 1 minimal erythema dose of UVB increases PAH activity for up to 24 h. The H_2O_2 generated by UVB radiation activates PAH, thereby playing a critical role in UVB-induced melanogenesis [42].

4.5. Dermal matrix

ROS have an established role in UV-induced skin aging, characterized by wrinkles. In general, wrinkles are created by alterations of the dermal matrix in which collagen levels are decreased by accelerated breakdown and collagen synthesis is reduced.

The $^1O_2$ generated by UVA irradiation stimulates the expression of matrix metalloproteinase (MMP)-1 in dermal fibroblasts through the secretion of interleukin (IL)-1α and IL-6 [43,44]. Oxidized lipids, such as linoleic acid hydroperoxide, also enhance the expression of MMP-1 and MMP-3 [45].

MMP-1 expression is stimulated by the activation of c-Jun N-terminal kinase, which is triggered by ROS after UV exposure. The activation of JNK is due to continuous phosphorylation of the epidermal growth factor receptor by ROS-dependent inactivation of protein tyrosine phosphatase [46]. An in vivo study showed that H_2O_2 accumulation in the skin due to a decrease in catalase also stimulates MMP-1 expression [47].

UV exposure of the skin also attenuates the synthesis of new collagen, which is regulated by activator protein (AP)-1 [48], due to a reduction of collagen synthesis modulated by ROS and effects on MMP-1 expression. In fact, exposure of human dermal fibroblasts to ROS also decreases collagen synthesis [49]. Furthermore, extracellular thioredoxin restores the reduction in collagen synthesis initiated by UVA/UVB and infrared radiation [50]. Thus, ROS also regulate collagen synthesis.

In the pathogenesis of scleroderma, which is characterized by excess collagen synthesis, ROS stimulates collagen synthesis. Fibroblasts from the skin of patients with scleroderma exhibit high levels of mRNAs encoding alpha1(I) and alpha2(I) collagens. In addition, they yield higher levels of $^{\bullet}O_2^-$ and H_2O_2 than do normal fibroblasts. N-Acetyl cysteine blocks the upregulation of collagen mRNA expression [51]. Furthermore, sufficiently high amounts of NO˙ increase collagen synthesis in dermal fibroblasts by stimulating heat shock protein 47, which is a molecular chaperone of collagen synthesis [52].

5. Effects of antioxidants on the skin and skin cells (Fig. 2)

5.1. Ascorbic acid

Ascorbic acid eliminates most ROS due to the oxidation of ascorbate to monodehydroascorbate and then to dehydroascorbate and has diverse functions to maintain the normal physiologic state in humans. In the skin, ascorbic acid is a cofactor required for the enzymatic activity of prolyl hydroxylase, which hydroxylates prolyl residues in procollagen and in elastin [53]. In addition, ascorbic acid is widely used as a depigmentation agent due to its inhibitory effect on tyrosinase. Recent studies reported newly discovered functions of ascorbic acid that contribute to the formation of the skin barrier by enhancing epidermal differentiation [54] and stimulating blood flow through NO˙ production via increases in the stability of tetrahydrobiopterin, a cofactor of constitutive NOS [55]. Heller et al. suggest that dark circles on the lower eyelid, which are caused by hyperpigmentation and poor blood circulation, are improved by ascorbic acid. In fact, in an in vivo study, ascorbic acid Na salt significantly improved dark circles due to effects on melanin, erythema, and dermal thickness [56]. These findings demonstrated the effects of ascorbic acid to suppress melanogenesis, to stabilize NOS, and to stimulate collagen synthesis.

Although ascorbic acid is widely applied to the skin to achieve these clinical improvements, its poor skin penetration and its instability in formulations reduce its clinical efficacy [57]. To overcome these disadvantages, several ascorbic acid derivatives, such as magnesium L-ascorbyl-2-phosphate [58], ascorbic acid 2-O-α-glucoside [59], 6-acylated ascorbic acid 2-O-α-glucoside [60], and tetra-isopalmitoyl ascorbic acid [61], have been synthesized and evaluated for their potential as pro-ascorbic acid derivatives.

5.2. Tocopherols (vitamin E)

Tocopherols are chemical compounds that comprise a chromanol ring and a hydrophobic side chain of an isoprene molecule,

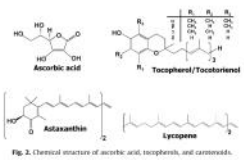


Fig. 2. Chemical structure of ascorbic acid, tocopherols, and carotenoids.

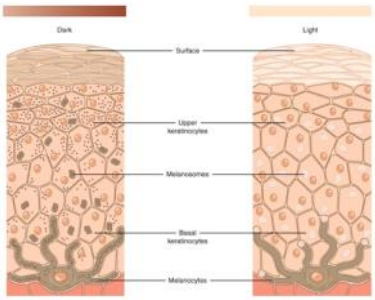
1. Ultraviolet (UV) light damages various components of the cell, like mitochondria and chromophores (which are molecules that give skin color - like melanin).
2. The UV light causes the loss of electrons, creating different reactive oxygen species (ex. $^3O_2$) which continue to form other versions of reactive oxygen species (ROS).
3. Without antioxidants, reactive oxygen species keep pulling electrons from nearby molecules and forming other reactive oxygen species - specifically leading to proteins have electrons pulled from them and fats/lipids having electrons pulled from them. This leads to protein carbonylation and lipid peroxidation, less useful forms of the original molecules and can lead to aberrant signaling within the cell. With antioxidants, these reactive oxygen species are neutralized.

Erythema: Reddening of the skin.

Stratum Corneum is another term for the **epidermis**, of the outside layer of skin. Oxidized proteins and lipids increase in the epidermis with UV exposure or winter (cold?).

Sebaceous Gland: A gland near the hair follicle in skin that releases oils to lubricate the skin and hair. Ultraviolet light (UV) stimulates the sebaceous gland to release more oil - this can induce an inflammatory process as acne forms or bacteria infiltrate, causing more ROS generation.

Melanogenesis
There can be an imbalance of different ROS types that can cause differences in reactions in the skin. Too much of one or too much of another can either increase the darkness or lightness of skin. This is caused, because melanocytes at the bottom layer of skin (made up of keratinocytes) produce melanosomes (organelles that produce melanin) and these melanin producing organelles (melanosomes) are released from the melanocyte to the keratinocytes. Melanin is a polymer (several amino acids/molecules stuck together) that darkens the cell (therefor darkening skin, overall). See Image.



Ascorbic Acid
This is Vitamin C. Innately, it gives up electrons, like other antioxidants and eliminates reactive oxygen species (ROS). However, it also plays a crucial function in hydroxylase reactions of collagen - meaning, it plays a role in increasing the stability of collagen fibers, thereby making them more heat resistant. It also reduces pigmentation by inhibiting a step in the formation of melanin - creating lighter skin. It is reported to help with dark circles around the eyes which are due to poor blood flow (it increases Nitric Oxide production). Pure forms of ascorbic acid are not easily absorbed by the skin, so modified versions have been synthesized for better absorption.

Dermal Matrix
Reactive Oxygen Species (ROS) induce collagen damage in the skin extracellular matrix that gives skin structure. Not only that, it reduces collagen synthesis and enhances the expression of metalloproteinases (MMP) from fibroblasts (skin support cells), leading to further breakdown of the collagen.

and are present in eight different forms based on the distinct substituted position of the methyl group in the chromanol ring and by the distinct unsaturation of the hydrophobic side chain. The antioxidative mechanism of tocopherols is partially due to the hydroxyl group in the chromanol ring donating a hydrogen atom to reduce free radicals.

Under physiologic conditions, α-tocopherol stimulates the GSH synthesis in HaCaT keratinocytes through the upregulation of γ-glutamylcysteine synthetase mRNA [62]. This finding suggests that tocopherol has biologic effects through the modulation of cellular responses.

Tocopherol has preventive effects in various oxidative stress conditions. 12-O-Tetradecanoylphorbol-13-acetate, which is a well-known tumor promoter, induces oxidative stress [63]. Application of tocopherol to the skin 30 min prior to treatment with 12-O-tetradecanoylphorbol-13-acetate inhibits the induction of H_2O_2, myeloperoxidase activity, xanthine oxidase activity, and lipid peroxidation [64]. α-Tocopherol acetate suppresses UVB-induced edema, erythema, and lipid peroxidation. UVA dramatically upregulates the expression of IL-8 mRNA and the secretion of IL-8 protein, and enhances AP-1 DNA binding activity. These effects of UVA are effectively reduced by α-tocopherol in a dose-dependent manner [65].

α-Tocopherol is expected to downregulate MMP-1 through its suppressive effects on AP-1 DNA binding. Dermal fibroblasts isolated from aged donors produce higher levels of MMP-1 than those from young donors. α-Tocopherol attenuates the increased collagenase gene transcription in aging fibroblasts without altering the level of its natural inhibitor, tissue inhibitor of metalloproteinase through the inhibition of protein kinase C α activity [66]. A detailed study of the ROS scavenging activity of tocopherols showed that γ-tocopherol is superior to α-tocopherol in its ability to scavenge $NO^{\cdot}$ [67]. Tocopherol, therefore, suppresses melanogenesis.

γ-Tocopherol is useful for suppressing melanogenesis and mRNA expression of tyrosinase and tyrosinase-related protein-2 in B16 melanoma cells [68]. A novel hydrophilic γ-tocopherol derivative was recently synthesized to reinforce its biologic effects. γ-Tocopherol-N,N-dimethylglycinate hydrochloride significantly reduces the formation of edema and tempered the increase in the COX-2-catalyzed synthesis of prostaglandin E induced by UV. Further, γ-tocopherol-N,N-dimethylglycinate hydrochloride strongly suppresses inducible nitric oxide synthase mRNA expression and $NO^{\cdot}$ production [69].

5.3. Carotenoids

Carotenoids are organic pigments that are naturally produced by plants, algae, some types of fungus, and some bacteria. β-Carotene and astaxanthin are components of carotenoids. In general, carotenoids possess the ability to quench $^1O_2$. Carotenoids are useful to protect against UV-induced damage. The mechanisms underlying the protective effects of carotenoids have been studied in a model of UVA-irradiated human dermal fibroblasts. Moderate doses of UVA stimulate fibroblast apoptosis; increase oxidative stress, including ROS generation; decrease antioxidant enzyme activities; promote membrane perturbation; and induce the expression of heme oxygenase-1. Among astaxanthin, canthaxanthin, and β-carotene, astaxanthin pre-loaded in fibroblasts protects against the UVA-induced alterations described above, indicating that astaxanthin has a superior preventive effect towards photo-oxidative changes in cell culture [70].

The lycopene concentration in skin also correlates significantly with skin roughness, suggesting that higher levels of antioxidants in the skin effectively decrease skin roughness, which is an early stage of wrinkle formation [71].

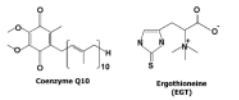


Fig. 3. Chemical structure of CoQ10 and ergothioneine.

5.4. Natural substances (Fig. 3)

Coenzyme Q10 (CoQ10) is also recognized as an intracellular antioxidative and energizing molecule, and reduces DNA damage triggered by UVA irradiation of human keratinocytes in vitro. CoQ10 suppresses MMP-1 production in dermal fibroblasts due to the downregulation of IL-6 expression in UVB-irradiated keratinocytes [72]. Furthermore, CoQ10 accelerates the production of basement membrane components, such as laminin 332 and type IV and VII collagens, in keratinocytes and fibroblasts, respectively; however, it has no effect on type I collagen production in fibroblasts. These findings suggest that CoQ10 has anti-aging effects through the accelerated production of epidermal basement membrane components [73].

Ergothioneine is a sulfur-containing amino acid presumed to function as a natural antioxidant. In cultured fibroblasts, ergothioneine suppresses the UVB radiation-induced upregulation of tumor necrosis factor-α. In addition, ergothioneine suppresses the expression of MMP-1 protein in fibroblasts exposed to UVA by quenching $^1O_2$ [74].

Zn(II)-glycine, a coordinated compound of Zn^{2+} and glycine, has A cell-membrane permeable inducer of metallothionein that protects against UVB-induced cell damage and suppresses IL-1α secretion and prostaglandin E2 synthesis in human normal keratinocytes [75]. In addition, Zn(II)-glycine not only reduces pro-MMP-1 production but it also reduces the MMP-1 in dermal fibroblasts induced by the conditioned medium of UVB-irradiated keratinocytes.

5.5. Polyphenols (Fig. 4)

Polyphenols are a group of chemical molecules produced in plants characterized by the presence of phenol units in their molecular structure. Epigallocatechin gallate (EGCG) is a representative polyphenol. Oral administration of EGCG for 8 weeks significantly increases the minimal erythema dose to UV and prevents disruption of the epidermal barrier function. These findings suggest that EGCG strengthens the tolerance of the skin to UV-initiating stress [76]. Furthermore, EGCG markedly reduces UVB-induced MMP-1, MMP-8, and MMP-13 in a dose-dependent manner, suggesting that EGCG attenuates the UVB-induced production of MMP via its interference with mitogen activated protein kinase-responsive pathways [77].

Recent studies on longevity have revealed the importance of SIRT1 and its activator [78], resveratrol, which is considered to be an important antioxidant. Resveratrol increases cell survival and concomitantly reduces ROS in UVB-exposed HaCaT keratinocytes. In addition, resveratrol suppresses the activation of caspases-3 and -8 in HaCaT cells [79].

Resveratrol prevents UV-induced skin aging through SIRT1 activation [80]. In addition, resveratrol directly inhibits tyrosinase activity and suppresses tyrosinase maturation, which decreases the pigmentation stimulated by the cAMP signaling pathway [81].

Tocopherols (Vitamin E)
This is Vitamin E. It stimulates the synthesis of an endogenous (self-created) antioxidant known as glutathione. Vitamin E decreases the UV-A stimulated increase in interleukin-8 (a pro-inflammatory protein/molecule). It decreases collagenase (breaks down collagen) gene transcription and is thought to potentially decrease metalloproteinases (MMPs) that degrade collagen, as well. It also, like Vitamin C, suppresses darkening of the skin (melanogenesis: darkening of skin).

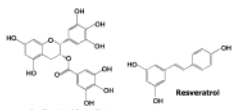


Fig. 4. Chemical structure of polyphenols, epigallocatechin gallate, and resveratrol.

6. Conclusions

Oxidative stress initiated by ROS generation is an important factor modulating skin alterations, especially those caused by UV exposure and aging. The human body has several endogenous oxidative stress-eliminating systems. Treatment with some antioxidants, such as ascorbic acid, tocopherols, and polyphenols, should be effective to enhance resistance to oxidative stress and prevent/improve skin aging. These findings will contribute to the development of future clinical and basic studies of the skin and potential treatments for skin diseases and deterioration with age.

Conflict of interest

None declared.

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