

Antioxidants and Skin Aging: A Review

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Skin aging is a complex, progressive, time-dependent deterioration caused by intrinsic (genetically programmed mechanisms) and extrinsic factors or environmental factors. These 2 processes are biologically different, but combined they lead to all of the cutaneous changes associated with aging skin. Skin is equipped with an elaborate antioxidant system that protects it from oxidative damage due to intrinsic and extrinsic factors. However, the natural antioxidant pool can be compromised or overwhelmed. Topical antioxidants have been demonstrated to protect the skin from damaging free radicals produced intrinsically by normal cellular metabolism or through exposure to UV light. In this article, we will discuss the different approaches used to prevent oxidative stress, induced skin aging through protection by topical as well as dietary antioxidants. Testing approaches for the different classes of naturally derived antioxidants and future research possibilities are discussed.

Skin, the largest organ of the human body, provides a major interface between the environment and the body. As the outermost organ, the skin is exposed to an array of chemical and physical environmental pollutants and is equipped with an elaborate system of antioxidant

substances and enzymes, including a network of redox-active antioxidants. However, the normal aging process, as well as environmental stress, can strip the epidermis of these protective antioxidants. An important component of environmental stress, UV irradiation is a potent generator of oxidative stress in the skin. Chronic exposure of human skin to UV irradiation increases the cellular levels of reactive oxygen species (ROS), which damage lipids, proteins, and nucleic acids in both epidermal and dermal cells and contribute to premature skin aging and photoaging.

Antioxidants constitute an important group of pharmacologically active agents capable of preventing the occurrence and reducing the severity of UV-induced skin damage and skin aging. This article focuses on contribution of oxidative damage in both extrinsic and intrinsic skin aging. Effects of both endogenously and exogenously supplied antioxidants in preventing skin aging and in vivo testing methodologies used to quantify the benefits of antioxidants in skin aging are discussed.

SKIN AGING: GENERAL CONCEPTS

Skin aging is a complex biological process that is a consequence of both intrinsic or genetically programmed aging that occurs with time and extrinsic aging caused by environmental factors.

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Intrinsic Versus Extrinsic Aging

Skin changes that accompany the normal process of aging are called *intrinsic* or *chronological aging*. Another way to think of this is biological (intrinsic) aging versus environmental (extrinsic) aging; the gene expression changes associated with these 2 types of aging differ.¹ In contrast, skin changes arising from external environmental causes is termed *extrinsic aging*.² A major cause of this extrinsic aging is the damage caused by UV radiation from the sun (photoaging).

Intrinsic (Biological) Aging

Intrinsic (biological) aging results from a combination of events including decreased proliferative capacity of skin-derived cells, decreased matrix synthesis in the dermis, and increased expression of the enzymes that degrade the dermal collagenous matrix. A sequence of events marks the onset of intrinsic aging. These events include: thinning and loss of much of skin's elasticity; flattening of the boundary between the epidermis and the dermis; loss of much of the cellular and extracellular matrix components of the dermis and the atrophy thinning of the dermal compartment; and reduction in the number of blood vessels in the dermis and the collapse of capillaries. In addition, hair follicles and sweat glands are reduced in number and function. Structural protein components such as collagen, elastin, and glycosaminoglycan, the major ground substance of the dermis, and organelles such as mitochondria, are reduced. Their functions and DNA repair and telomere structure maintenance decline. Hormonal changes and balance also occur with chronologic aging.²

Extrinsic (Environmental) Aging

Extrinsic (environmental) aging of skin is a process caused by external factors, including UV radiation, cigarette smoking, air pollution, and exposure of skin to other environmental factors such as cold, heat, dust, and smog. Lifestyle choices such as diet, exercise, and sleep habits, as well as stress and diseases, also impact aging. The effects of unchecked oxidative stress include dysfunction of cells, mitochondria, electron transport, ribosomes, DNA, telomere repair, inflammation, cell membrane damage, immune dysfunction, and cancer. Of all extrinsic causes, UV radiation from sunlight has the most negative effects on the skin, although less so on skin of color. Because of this, extrinsic aging is often referred to as photoaging.³

Photoaging is a slow process resulting from chronic exposure to solar UV radiation. The degree of photoaging is determined by the skin type and by the total lifetime sun exposure. Episodic photoaging will eventually culminate in permanent photoaging.⁴ In photoaged skin,

the epidermis becomes less elastic and more fragile. The small blood capillaries in the dermis decrease in number, and the remaining blood vessels become tortuous and dilated. The elastic fibers degenerate, producing a thickened mass that replaces the collagen (elastosis). Severely photodamaged skin results in warty or hyperkeratotic spots on the skin, called *actinic keratoses*, which could eventually develop into skin cancer.⁵

Daily, routine exposure to the sun, even in small doses, can lead to long-term effects that accumulate with time. Most damaging to human skin is UVB of the solar spectrum (290–320 nm). However, some exposure to UVB (eg, 15 minutes of evening sun) is necessary for adequate vitamin D production in the skin. Although less damaging than UVB, UVA (320–400 nm) is measured by sunburn (erythema) or damage to cell DNA, and penetrates skin deeper, causing damage to the dermis. UVA1 (340–400 nm) may be particularly damaging; UVB and UVA1 produce different changes in the gene expression and clinical appearance of photoaging. In addition, 20 times more UVA reaches the earth in the middle of a summer's day than UVB. In addition, UVA contributes significantly to the total exposure, and since it penetrates deeper into the skin, it leads to deeper damage than UVB.⁶

ANTIOXIDANTS AND SKIN AGING

Oxidative damage initiated by ROS is a major contributor to skin aging. Free radicals generated internally within the skin cell during normal oxidative metabolism or by external sources such as UV irradiation are major contributors for skin aging. Skin exposure to ionizing and UV radiation generates ROS in excessive quantities that can deplete tissue antioxidants and other oxidant-degrading pathways. Uncontrolled release of ROS is involved in the pathogenesis of a number of human skin disorders, including premature skin aging.⁷ In addition to UV radiation, heme pathway intermediates may have pro-oxidant effects, whereas heme oxygenase, an enzyme that degrades heme, can function as both an antioxidant and a pro-oxidant.⁸ Exposure of skin to UVA releases labile iron, leading to oxidative stress in skin.

ANTIOXIDANT PROTECTION FOR SKIN AGING

Human skin is equipped with an array of antioxidant enzymes to protect the cells from damaging effects of free radicals. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH) biosynthesizing enzymes protect the tissues from free radicals. In addition, antioxidant molecules such as vitamins A, C, and E slow the process of aging either by preventing free radicals from oxidizing sensitive biological molecules or by reducing

the formation of free radicals and quenching the already formed ROS. The levels of these antioxidants, as well as antioxidant enzymes, are reduced by age and various environmental stressors, such as UV exposure. Replenishing these antioxidants either by topical application or by dietary ingestion can protect skin from aging. The level of dietary or topical antioxidants achieved in the skin varies with the individual antioxidant and also with absorption and other factors. In the following section, we describe different antioxidant systems relevant for skin biology and antiaging benefits.

ENDOGENOUS ANTIOXIDANTS IN SKIN

A complex regulation of the antioxidant defense system during intrinsic aging and photoaging processes has been described.⁹ The activities of catalase and GSH reductase increased in the epidermis of photoaged and naturally aged skin. The concentrations of α -tocopherol, ascorbic acid, and GSH were generally lower in the epidermis of photoaged and aged skin. Uric acid did not show any significant changes. In general, a gradient of antioxidants with high levels in the basal layers and lower levels in the upper layers of the epidermis has also been demonstrated.¹⁰ Thus, a normal aging process as well as UV stress can deplete the antioxidant system of the skin, leading to ROS generation and oxidative damage to proteins, lipids, and nucleic acids in the skin. Therefore, it is important to supplement skin with natural antioxidants as well as plant-derived antioxidants to provide skin protection.

Antioxidant molecules in the skin interact with ROS or their by-products to either eliminate them or to minimize their deleterious effects.¹¹ These antioxidant molecules include GSH, α -tocopherol or vitamin E, ascorbic acid or vitamin C, GSH peroxidases, GSH reductase, GSH S-transferases, SODs, catalase, and quinone reductase. Ascorbic acid and GSH are soluble antioxidants, whereas vitamin E is membrane bound and capable of intercepting free radical-mediated chain reactions.⁷ Administration of agents that augment tissue GSH levels, such as N-acetylcysteine, provide protection against the toxic effects of ROS-generating agents.¹² In aged human skin, SODs, copper and zinc SOD, and manganese SOD are decreased.¹³ Enhancing the activities of these antioxidant enzyme systems by providing their metal cofactors such as copper, manganese, zinc, and selenium can boost their activities and provide better antioxidant protection for skin.

COMMON ANTIOXIDANTS WITH ANTIAGING BENEFITS USED FOR SKIN

Ascorbic Acid

Ascorbic acid (vitamin C) is one of the most important antioxidants that protects the skin and prevents skin

aging and photoaging.⁵ In cells, it is maintained in its reduced form by reaction with GSH. In addition to its direct antioxidant effects, ascorbic acid is also a cofactor involved in collagen synthesis in humans. Therefore, its deficiency results in diseases of the skin, gums, and other tissues with high collagen content.

The richest natural sources for vitamin C are fruits and vegetables. Among fruits, citrus fruits and berries contain the highest concentration of the vitamin. It is also present in some cuts of meat, especially liver. Vitamin C is the most widely taken nutritional supplement and is available in a variety of forms, including tablets, drink mixes, crystals in capsules, or naked crystals. The most common topical and dietary source of vitamin C is made synthetically from glucose, mainly using a fermentation process.

Tocopherols and Tocotrienols (Vitamin E)

Vitamin E is the collective name for a set of 8 related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α -tocopherol has been most studied, as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form.¹⁴ The most important lipid-soluble antioxidant is α -tocopherol, which protects membranes from oxidation by reacting with lipid radicals produced in the lipid-peroxidation chain reaction.

High levels of vitamin E are found in many foods, such as asparagus; avocado; nuts (eg, almond and hazelnut); oils (eg, red palm, canola, sunflower seed, and olive); leafy green vegetables such as spinach; wheat germ; and whole grains. Animal sources are eggs and milk. Most of the vitamin E that is used in dietary supplements and topical use is derived synthetically as α -tocopherol.

Lipoic Acid

Lipoic acid is an organo-sulfur compound, which is an essential cofactor for many enzyme complexes. One of the most visible roles of lipoic acid is as a cofactor in aerobic metabolism, specifically the pyruvate dehydrogenase complex. Dihydrolipoic acid, the reduced form of lipoic acid, is a potent antioxidant. It is able to regenerate (reduce) antioxidants, such as GSH, vitamin C, and vitamin E.¹⁵

Lipoic acid is not seen in natural foods such as fruits or vegetables. It is normally synthesized in the animal body. Animal liver and yeast are good sources of lipoic acid. Lipoic acid is synthesized from foods rich in sulphur containing amino acids such as cysteine and methionine. Most, if not all, of the lipoic acid supplements used in dietary as well as topical products are produced synthetically.

Ubiquinone (Coenzyme Q10)

This oil-soluble vitaminlike substance is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of adenosine 5'-triphosphate. Because of its ability to transfer electrons, it also acts as an antioxidant.¹⁶

Like lipoic acid, ubiquinone does not occur in significant amounts in fruits or vegetables; it is synthesized in animal tissues. The richest natural source is the red tissue of mackerel and herring. It is also found in the livers of animals. All of the commercial sources of ubiquinone used in topical products are synthetically derived.

Carotenoids (Lycopene, Lutein/Zeaxanthin, Beta-Carotene)

Carotenoids belong to the category of tetraterpenoids (ie, they contain 40 carbon atoms). Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls. The unoxygenated (oxygen-free) carotenoids such as alpha-carotene, beta-carotene, and lycopene are known as carotenes. Carotenoids are efficient free-radical scavengers and have antioxidant activity.¹⁷

Carotenoids contribute to the orange and red color of many different fruits and vegetables. Crude palm oil, yellow and orange fruits (eg, mangoes and papayas), orange root vegetables (eg, carrots and yams), and green leafy vegetables (eg, spinach, kale, sweet potato leaves, and sweet gourd leaves) are rich sources of carotenoids. Many such colored fruit extracts used in topical skin care products contain rich sources of carotenoids.

Plant-Derived Polyphenols

Polyphenols are a group of chemical substances found in plants characterized by the presence of more than 1 phenol unit or building block per molecule. Polyphenols are generally divided into hydrolyzable tannins (gallic acid esters of glucose and other sugars) and phenylpropanoids, such as lignins, flavonoids, and condensed tannins. The largest and best-studied polyphenols are the flavonoids, which include several thousand compounds, among them the flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids.¹⁸ Polyphenols can also be esterified, methylated, or polymerized, creating a new class of polyphenols. For example, ellagic acid is a dimer of gallic acid and forms the class of ellagitannins, or a catechin and a gallo catechin can combine to form the red compound theaflavin, a process that also results in the large class of brown thearubigins, in tea. Dozens of polyphenols have been shown to have antioxidant, and anti-inflammatory activities on skin and are currently used in skin care products.

High levels of polyphenols are generally found in fruit skins. Notable sources of polyphenols include berries, tea, beer, grapes and wine, olive oil, chocolate and cocoa, coffee, walnuts, peanuts, pomegranates, and other fruits and vegetables. Many natural extracts used in cosmetics and topical products are rich sources of polyphenols.

RECENT DEVELOPMENTS IN ANTIOXIDANTS FOR SKIN BENEFITS Resveratrol

Trans-3,5,4'-trihydroxystilbene, belongs to a class of polyphenolic compounds called stilbenes. In addition to being a powerful antioxidant, resveratrol has multiple biological activities, including anti-inflammatory, antitumorigenic, estrogenic, cell-cycle regulation, apoptotic induction, and induction of antioxidant enzyme systems. Resveratrol has been claimed to be beneficial for cardiac health, cancer, longevity (via inducing a caloric restriction-like effect), and skin health.¹⁹

The richest natural sources of resveratrol are grapes, peanuts, mulberry, blueberry, and bilberry. The amount found in grape skins also varies with the grape cultivar, its geographic origin, and exposure to fungal infection. Red wine is a rich source of resveratrol; the amount of fermentation time a wine spends in contact with grape skins is an important determinant of its resveratrol content. Most of the resveratrol used as dietary or topical supplements is extracted from Japanese knotweed.

Green Tea Polyphenols

Green tea contains polyphenolic compounds also known as epicatechins, which are powerful antioxidants and anti-inflammatory compounds. The major and most reactive constituent in green tea responsible for these biochemical or pharmacological effects is (-)-epigallocatechin-3-gallate. Treatment of skin with green tea polyphenols has been shown to modulate the biochemical pathways involved in inflammatory responses, cell proliferation, and responses of chemical tumor promoters, as well as UV light-induced inflammatory markers of skin inflammation. Many cosmetic companies have exploited these properties of green tea polyphenols and use them in products for their antiaging benefits.²⁰

The richest source of polyphenols is green tea, followed by black tea. The polyphenol content of green tea varies depending on the type of tea and brewing time and temperature. Green tea polyphenols used in the cosmetics and dietary supplement industries are extracted from green tea.

Caffeine, Caffeic Acid, Quinic Acid, Chlorogenic Acid, and Ferulic Acid

Found in grains, fruits, and vegetables, caffeine, caffeic acid, quinic acid, chlorogenic acid, and ferulic acid are

powerful antioxidants and protect skin from UV-induced damage. Ferulic acid is used as a photoprotective agent in combination with other antioxidants such as vitamin C.²¹ A botanical extract from whole coffee cherry contains a mixture of these potent natural plant polyphenol antioxidants and also has photoprotective effects.

The most abundant source is grains, fruits, and coffee seeds. Commercial sources are extracted from coffee seeds and the fermentation process.

Quercetin

One of the most abundant flavonoids, present in fruits, vegetables, and beverages, is quercetin, a powerful antioxidant and iron chelator. Quercetin, in addition to having its own antioxidant activity, also protects skin antioxidant systems and prevents UV-induced skin photodamage.²²

Foods rich in quercetin include capers, apples, tea (*Camellia sinensis*), onion (especially red onion), red grapes, citrus fruit, tomato, broccoli and other leafy green vegetables, and a number of berries, including cherry, raspberry, cranberry, and the fruit of the prickly pear cactus. Commercially used quercetin is extracted from natural sources.

Phloretin

Phloretin, a dihydrochalcone polyphenol, together with its glucoside phloridzin, are unique compounds found in apples and are thought to be important in the health-promoting effects of this fruit.²³ Phloretin has strong antioxidant effects when tested against experimental stable free radicals,²⁴ hydroxyl radical,²⁵ and prevention of lipid peroxidation.²⁶ Phloretin inhibits matrix metalloproteinase (MMP)-1 and elastase,²⁷ enzymes that degrade connective tissue and are considered important in photoaging. Phloretin is used as a photoprotective agent in combination with other antioxidants, such as vitamin C.²¹

The richest source of phloretin is apple skin. It is extracted from apple skin and used in topical products.

Genistein and Other Soy Flavonoids

Genistein and related flavonoids seen in soybeans have a variety of properties, including antioxidant, anti-inflammatory, estrogen-mimetic, and cell-cycle-regulating.⁸ Topical application of genistein and its metabolites or derivatives, such as daidzein, inhibited UVB-induced ROS generation and contact hypersensitivity and reduced inflammation.

The most abundant source of soy flavonoids is soy extract. Topical products containing soy extracts have high levels of genistein and other flavonoids.

Nordihydroguaiaretic Acid

Nordihydroguaiaretic acid, a polyphenolic compound from the bush *Larrea tridentata* (creosote bush), is a powerful

antioxidant and anti-inflammatory with multiple photoprotective activities in skin.²⁸

Nordihydroguaiaretic acid is found in high amounts in the long-living creosote bush. Another rich source is the chaparral bush, a shrub found in the desert regions of the southwestern United States and Mexico. It is not commonly used in cosmetics or topical products.

Rosmarinic, Ursolic, and Carnosic Acids

Found as major components of rosemary and sage, these compounds have multiple activities including anti-inflammatory, antioxidant, and chemoprotective effects against UV and carcinogens. Rosemary and sage extracts have been used in cosmetic products for antiaging benefits.²⁹

These pentacyclic triterpene compounds are present in many plants, including apples, basil, bilberries, cranberries, elder flower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, and prunes. Apple peels contain a high quantity of ursolic acid and related compounds. These compounds have excellent properties as anti-inflammatory agents in topical products.

Silymarin

A component of milk thistle, silymarin and its derivatives have been traditionally used in European medicine, mainly for the treatment of liver diseases. Topical application of this compound reduces UV-induced erythema and edema and provides photoprotection from oxidative stress because of its anti-inflammatory and antioxidant properties.²²

Curcuminoids

The human health benefits of curcumin and its derivatives from the root *Curcuma longa*, a spice found in curry powder, are widely known. Curcumin has numerous properties, including antioxidant, anti-inflammatory, anticancer, and photoprotective, as well as the potential to treat a wide variety of inflammatory diseases such as cancer, diabetes, cardiovascular diseases, arthritis, Alzheimer disease, and psoriasis, among others, through modulation of numerous molecular targets. It has been used in skin care products for photoprotection, skin-lightening effects, and general antiaging benefits.³⁰

Isothiocyanates (Sulforaphane)

Present in broccoli sprouts and recently discovered for their powerful anti-inflammatory properties, isothiocyanates have been proposed to be useful as protective compounds for skin protection against UV-induced photodamage.³¹

Other Phytoextracts

Various phytoextracts with antioxidant properties have been used in recent years in cosmetic formulations for

antiaging benefits. These phytoextracts include: *Ginkgo biloba*, ginseng, soy, tea tree oil, *Arnica*, bromelain, chamomile, pomegranate, licorice, CoffeeBerry, açai, and grape seed extracts. The list of these antioxidant and anti-inflammatory extracts is growing longer with the addition of new and exotic plant extracts from various parts of the world, including traditional Chinese medicine, Indian Ayurvedic medicine, and African and Amazonian traditional medicine, and extracts from remote mountainous regions.²²

COMPLEMENTARY EFFECTS OF ANTIOXIDANTS AND SUNSCREEN ON SKIN AGING

Since UV radiation is one of the major causes of skin damage, protection of skin by the use of a physical or chemical sunscreen is highly recommended. However, sunscreens contain chemicals that work only on the surface of the skin to absorb or reflect certain wavelengths of radiation. Organic sunscreen chemicals are limited by the wavelengths that they can absorb. No sunscreen fully protects against all UV wavelengths. Physical sunscreens such as zinc oxide and titanium dioxide reflect, scatter, and block UV radiation (often depending on particle size). Protective clothing also may be used to block UV light. Regular window glass blocks UVB but not UVA1 wavelengths. Sunscreen protection is also a function of the uniformity of the application to the surface of the skin. Sweating and rubbing can alter the uniformity and provide holes through which UV light may penetrate. After removal, the sunscreen must be reapplied to be effective. When some sunscreens absorb energy, they are chemically destroyed and no longer efficient; thus, periodic reapplication is required. Physical sunscreens are not chemically destroyed, but they do present formulation challenges due to their whiteness on the skin. For all of these reasons, sunscreens are recommended to be reapplied regularly.

Antioxidants protect the skin from the inside by neutralizing ROS generated by UV radiation. Antioxidants are present at the site of the initial ROS-mediated injury or reaction. They can neutralize the oxidative stress and prevent the chemical reaction from happening. In the course of the reaction, the antioxidant is depleted. In time, the antioxidant capacity of skin may become inadequate, and damage will ensue. Therefore, topical antioxidants formulated to enter the skin can add to the skin's own antioxidant pool and increase protection. These topical antioxidants have an advantage over sunscreens because once inside skin they are stable, cannot be removed by washing or rubbing, and last for several days.⁵

CLINICAL PROTOCOLS TO MEASURE ANTIOXIDANT PROTECTION

Several protocols have been adapted for testing the effects of antioxidants for the prevention of skin aging.

Protective Role of Antioxidants to Prevent Skin Photoaging

The effects of a single topical antioxidant or mixtures of antioxidants on attenuating the harmful effects of UV irradiation can be studied using normal, healthy participants. In a typical protocol, 10 to 20 participants (aged 18–60 years; Fitzpatrick skin types II and III) will be randomized and treated with an antioxidant product or vehicle control on the lower back for 4 consecutive days.²¹ On day 3, the minimal erythema dose (MED) is determined for each participant at a different site on the back. On day 4, the 2 test sites receive solar-simulated full-spectrum UV irradiation at 1 to 5 times the MED at intervals of 1 times the MED. (Testing with only UVB or only UVA1 is also possible.) On day 5, photographs for colorimetric evaluation as well as punch biopsies will be obtained from the antioxidant-treated area, the vehicle-treated area, and from an adjacent area that received no UV radiation or treatment. The biopsies can be used for immunohistochemistry or other immunological methods to evaluate specific biomarkers. In general, biomarkers include, but are not limited to, increases in sunburn cell formation, thymine dimer formation, MMP-9 expression, and p53 protein expression. Other potential UVA and UVB biomarkers include: inflammation markers such as interleukins and their receptors,³³ protein oxidation markers,³² or other noninvasive markers such as skin autofluorescence changes.³⁴ Effects of UV irradiation on immunosuppression can also be quantitated by evaluating specific Langerhans cell markers. UV irradiation is known to suppress the amount of CD1a-expressing Langerhans cells. Pretreatment of skin with the antioxidant composition can block this effect.²¹

Antioxidants for Protecting Chronological Skin Aging

The effect of long-term treatment of antioxidant compositions on protecting skin from chronological aging or reversal of aging symptoms can be studied in long-term studies lasting between 3 and 6 months. Typically, these studies will be multicenter clinical studies recruiting women aged 35 to 65 years with mild to moderate periorcular fine and coarse wrinkles and mild to moderate hyperpigmentation on the face and hands. The antioxidant composition will be used once daily on the face, chest, neck, and the back of both hands. Assessments will be done at baseline (pretreatment) and weeks 4, 8, 12, 18, and 24 posttreatment.

At each study visit, the clinician will grade the parameters using a 10-point scale. Objective and subjective irritation parameters will be evaluated using a 4-point scale, where 0=none and 3=severe. Bioinstrumental evaluation will include Chroma Meter measurements of the hyperpigmented lesions on the face and elasticity meter (eg, Cutometer, BTC-2000) measurements for the skin's viscoelastic properties. Silicone replicas or digital profilometry (eg, PRIMOS) of the periorcular wrinkles will be taken at each study visit. Digital images of the face (frontal, left and right sides) will be taken to evaluate efficacy. Self-assessment questionnaires will also be used to evaluate the perceived efficacy by the participants. Classic skin biomarkers that change with age, such as collagen-1/3, MMPs, and extracellular matrix components such as glycosaminoglycans, syndecans, and CD44, may also be evaluated using immunohistology on skin biopsies done at baseline and at 3 and 6 months with the antioxidant formulation.

In Vivo Measurement of Antioxidant Benefits

The in vivo ROS detection in skin with and without UV stimulation has recently developed as a result of evaluating antioxidants in vivo under physiological conditions and over time.³⁵ Recent in vivo studies have reported successful evaluation of ROS and scavenging via fluorescence measurements on tape strip layers for the assessment of skin's natural antioxidant defense capability³⁶ and the efficacy of antioxidants toward UVA-induced radicals via fluorescence on D-squames from human participants.³⁷ Treatments can be applied before skin exposure to UV light on human participants under controlled environmental and experimental conditions. Photogenerated under UVA, ROS are generally expected to be singlet oxygen, hydroxyl radicals, hydrogen peroxides, or lipid peroxides. Fluorescent probes can then be used to reveal the presence of the ROS on tape strips of stratum corneum to avoid direct contact of these toxic reagents to human skin. Depending on the molecular probe, radicals are quantified via a specific fluorescence band by accounting for the amounts of corneocytes present on each strip via UV-transmittance measures. Some ROS have extremely short half-lives and are difficult to measure. Comparative studies have shown radical scavenging efficacies of different antioxidants sensitive to 1 or several ROS, but the method limits their evaluation to radicals present or generated in the stratum corneum.

FUTURE TRENDS IN ANTIOXIDANT RESEARCH AND SKIN AGING

A standardized method to characterize and compare the properties and oxidative stress protection capacity

of antioxidants is still lacking. Various investigators have used a variety of in vitro and in vivo methods to quantify the benefits of antioxidants on human skin. In a recent development, a multistep in vitro process utilizing a variety of biochemical and cell biological methods combined with in vivo studies was designed to compare the oxidative stress protective capacity of commonly used antioxidants.³⁸ Using this method, the benefit of a novel bioengineered antioxidant, idebenone, has been determined in comparison to the classic antioxidants such as vitamins C and E and ubiquinone. New in vivo and in vitro testing methods to evaluate the content as well as the efficacy of antioxidants on human skin are constantly being developed.

Recent trends in the synthesis and evaluation of novel antioxidants that are more stable, more bioavailable, and more skin penetrable have resulted in many new antioxidant derivatives. One such example is idebenone, which is a more stable and potent analogue of coenzyme Q10. Idebenone has been shown to be more stable, more bioavailable, and more potent in clinical studies.³⁹ In addition, recent improvements in the topical skin care delivery of idebenone via a new synthetic molecular derivative (the di-palmitic acid ester of idebenone) has further enhanced this antioxidant technology, providing increased skin and cell permeability and improved time-released action.

A water-soluble derivative of resveratrol (resveratrol triphosphate) demonstrated a more homogeneous distribution of resveratrol throughout the stratum corneum and viable epidermis.⁴⁰ New bioconjugates of resveratrol with lipoic acid and vitamin E have been synthesized to improve their photostability and to modulate their lipophilic character. These complexes are hydrolyzed by a combination of stratum corneum enzymes and the cholesterol esterase.⁴¹ Other novel phytoextracts with better antioxidant activities are constantly being developed. New synthetic antioxidants with the ability to boost the effects of sunscreen are being developed to provide UV protection from the outside as well as antioxidant protection from the inside of the skin.⁴²

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