

Free Radicals and Aging of the Skin

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This article examines the molecular mechanisms involved in photoaging and reviews topical antioxidant agents that have inundated the commercial market with claims of providing potent antioxidant effects on the skin. The article presents some of the scientific information available regarding these agents and provides an overview of the basic process of skin aging.

The central role of free radicals in skin aging has resulted in the marketing of many antioxidants and has had major effects in financial markets. The scientific basis for many of these antioxidants lags behind their promotion. Skin aging can be divided into 2 major categories: intrinsic aging and extrinsic aging or photoaging.

Intrinsic aging is defined as the natural or chronologic aging of skin, excluding that caused by outside variables such as sunlight or chemicals. Clinically, with intrinsic aging, skin becomes loose or lax, thin, and different in pigment and tone. These clinical changes can be easily explained through histologic examination of tissue. There are changes in epidermal and dermal cellularity, thinning of rete ridges, degeneration of the dermal matrix (ie, collagen, elastin), loss of glycosaminoglycans, and disorganization of microvasculature.¹ There are 2 major theories of intrinsic aging. In the first, intrinsic aging is thought to be caused by genetic programming; in the second, intrinsic aging is the result of wear and tear on the system. According to the latter theory, natural cellular processes lead to the formation of free radicals, such as singlet oxygen, superoxide, and hydroxyl ions, which in turn react with DNA, proteins, and cell surface molecules, causing cell damage in the process. Over time, repair systems become faulty, leading to cell death or damage.²

Extrinsic aging is caused by the effects of external factors on the skin. These factors include ultraviolet radiation (UVR), chemicals, and smoking. As UVR is the most significant of these factors, extrinsic aging has been termed *photoaging*. Clinically, with photoaging, skin takes on a leathery appearance, wrinkling becomes prominent, splotchy discoloration and dyspigmentation become evident, and tumors, both benign and malignant, appear. Histologic correlation reveals a relative thickening of the epidermis initially and then eventual atrophy of the epidermis with loss of normal cellular

architecture and cellular atypia.^{3,4} Additionally, in the dermis, there is alteration of functional fibroblasts, degeneration of dermal collagen and elastin, deposition of elastotic material, and deterioration of dermal blood vessels. Early research focused on elastin, but a growing body of evidence suggests that reactive oxygen species (ROS) are generated by UVR, resulting in oxidative damage to cellular components such as mitochondria as well as nuclear DNA damage, which in turn accelerates aging and contributes to skin cancers.⁵ Interaction between ROS and collagen leads to altered metabolism of collagen, degradation of proteoglycans, and decreased synthesis of proteoglycans.² Fisher and Voorhees⁶ presented a working model for the pathophysiology of photoaging. They suggested that UVR activates cell-surface growth factors and cytokine receptors in keratinocytes and fibroblasts. Receptor activation results in signal transduction through protein kinase cascades that activate the transcription factor, AP-1, in the nucleus. UVR-activated AP-1 stimulates transcription of matrix metalloproteinase (MMP) genes that encode for collagenase, gelatinase, and stromelysin. These MMP genes degrade collagen and extracellular matrix. Activation of AP-1 is thought to occur through activation of cell-surface receptors by ROS. AP-1 is a complex of c-Fos and c-Jun heterodimers and other factors. Studies have shown that only c-Jun is induced by UVR; therefore, AP-1 activation depends primarily on c-Jun induction. In addition to inducing AP-1-mediated MMP, UVR induces endogenous tissue inhibitor of MMP (TIMP).⁶ This enzyme suppresses MMP activity, thereby preventing excessive breakdown of connective tissue.

The skin has evolved a number of mechanisms, both enzymatic and nonenzymatic, to deal with these potentially damaging ROS. The enzyme systems include superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, and catalase.⁷ The nonenzymatic mechanisms include a number of endogenous antioxidants within the epidermis and dermis. These include, in order of concentration, vitamin C, glutathione, vitamin E, ubiquinone, and α -lipoic acid.⁷

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These endogenous antioxidants, along with free radical-scavenging enzyme systems, remove deleterious ROS. Over the years, researchers have studied the effects of a number of exogenous agents that may reduce, inhibit, and even reverse UVR-induced damage to the skin. The rationale that topical antioxidants prevent or reverse photoaging is based on the fact that UVR depletes cellular antioxidants, leading to oxidative stress, leading to damage to cellular components. Antioxidants prevent or reduce oxidative stresses by removing damaging free radicals. Although sunscreens are the agents most widely used for photoprotection, they are not discussed here. Needless to say, any therapeutic regimen for reversing or preventing sun damage should include daily use of sunscreens. This article covers the commonly used topical antioxidants: retinoids (all-trans-retinoic acid [ATRA] or tretinoin), vitamin C, and vitamin E.

TREATMENT OF PHOTOAGED SKIN

Retinoids

The retinoids, a group of compounds structurally related to vitamin A, exert a number of effects on the skin, including effects on keratin synthesis, sebaceous gland activity, and proliferation and terminal differentiation of fibroblasts. The retinoids exert their effects through retinoic-acid receptors (RARs, RXRs) in the nucleus. Both RARs and RXRs are found in the skin. Through binding of specific heterodimers, activation of selective receptors occurs, producing specific actions. Most of the effects are exerted through nuclear changes in transcription or translation of gene products.

In 1986, Kligman et al⁸ showed that topical application of ATRA improved the clinical appearance of photoaged skin through a reduction in fine wrinkling and dyspigmentation. They also reported histologic improvement or reversal of UVR-induced changes, as demonstrated by thickening of the previously atrophic epidermis, disappearance of cellular atypia, normalization of the cytologic architecture, formation of new collagen, formation of new vasculature, and disappearance of elastotic material from the dermis. Several studies have confirmed that tretinoin induces long-term reversal of UVR damage by increasing collagen synthesis, decreasing abnormal elastin deposition, and normalizing glycosaminoglycan formation. Although the efficacy of tretinoin in reversing photodamage is firmly established, the mechanism by which this occurs is still being studied. Tretinoin binds to RARs, which in turn bind to regulatory regions within the DNA, resulting in downstream signal transduction through activation of MAP kinase pathways.^{6,9} It seems that pretreatment with ATRA in cultured cell inhibits UVR induction of c-Jun protein translation, resulting in inhibition of UVR-induced upregulation of MMP genes.⁹ Interestingly, ATRA does not inhibit UVR induction of TIMP.⁹

This inhibition of MMP genes results in suppression of UVR-induced collagen breakdown and normal TIMP activity.

Long-term treatment with tretinoin results histologically in increases in dermal collagen, decreases in abnormal elastin, increases in mucin, and decreases in melanin. Clinically, use of ATRA decreases wrinkling, alters UVR dyspigmentation, decreases the number of sun-induced keratoses, and improves the overall appearance of the skin.^{8,10} Early studies were performed with tretinoin at high concentrations (eg, 0.1%); the resulting irritation was suggested as contributing to the effects observed.⁸ Later studies proved that lower concentrations (eg, 0.025%) had similar beneficial effects on photoaging without irritation.¹¹ Although most studies have involved tretinoin, other vitamin A derivatives have been shown to lessen the effects of photoaging.¹²

Many physicians recommend combining alpha hydroxy acids (AHAs) and tretinoin to enhance clinical improvement of photoaged skin, but no published reports support this practice. Further studies are needed to establish the benefit of combined use of AHAs and tretinoin in treating photoaged skin.

Vitamin C

L-ascorbic acid is the active form of vitamin C and is the most abundant antioxidant in the skin.⁷ Humans cannot synthesize vitamin C and therefore must rely on dietary supplementation. L-ascorbic acid is water-soluble and serves as the major aqueous-phase reductant in the body. Besides having antioxidant properties, vitamin C is required for collagen synthesis, norepinephrine synthesis, tyrosine catabolism, and activation of a number of peptide hormones.¹³ The minimal daily allowance for vitamin C, 60 mg/day, may be inadequate for providing the skin with sufficient levels of protection against the oxidative stresses of UVR. If antioxidants could be applied directly to sites of damage, photoprotection could be improved. A number of topical vitamin C preparations have been touted as offering photoprotection. The challenge, however, has been to develop a stable aqueous L-ascorbic acid preparation that is absorbed percutaneously and that provides continuous UVR protection. Absorption studies have demonstrated that the free acid form is absorbed through the skin. Because the pK_a is 4.2, absorption of the free acid is maximal at a pH less than the pK_a .¹⁴ Pinnell et al¹⁴ demonstrated that daily application of 15% to 20% aqueous preparations at pH 3.5 resulted in saturated skin concentrations of L-ascorbic acid and that these concentrations lasted for 4 days and provided significant protection against photodamage. Topical L-ascorbic acid has been shown to decrease UVB erythema in humans and psoralen-UVA phototoxicity in pigs and to inhibit development of sunburn.¹³ Photoprotection by topical L-ascorbic acid may be more than that provided by sunscreens alone for

several reasons. First, topical L-ascorbic acid, once absorbed, cannot be washed off the skin and remains in the skin for as long as 4 days, providing a reservoir of antioxidant. Second, topical L-ascorbic acid absorbs both UVB and UVA. Additionally, topical L-ascorbic acid improves collagen synthesis and is an anti-inflammatory agent. Vitamin C prevents UVR-induced immunosuppression, lightens skin through inhibition of tyrosinase, and has been used to treat sunburn.¹⁴

Despite the immense popularity of topical vitamin C products and the scientific data regarding the effects of antioxidants (eg, topical vitamin C) on in vitro photoaging, very few, if any, clinical trials studying in vivo effects have been reported. Vitamin C is potentially synergistic or additive with retinoids, but studies are needed to evaluate this potential.

Vitamin E

Vitamin E is the most abundant endogenous lipid-soluble antioxidant, with α -tocopherol being the most biologically active form. There is a synthetic form of vitamin E derived from phytol—all-rac- α -tocopherol (dl- α -tocopherol). The d-isomer seems to have more relative potency than the other isomers.¹⁵ The antioxidative properties of α -tocopherol are closely linked with its regeneration by other antioxidants, such as glutathione and L-ascorbic acid. These latter agents are the major cofactors of vitamin E in protecting against oxidative damage. Ubiquinone (ie, coenzyme Q) may also be a biological regenerating agent for vitamin E. The mechanism of action of vitamin E seems to involve free-radical chain propagation of lipids. UVR increases the number of ROS, which in turn react with cell-surface and cellular lipids, generating highly reactive lipid peroxides (LOOHs). These LOOHs damage the membrane by reacting with other lipids, thus propagating free radicals. α -tocopherol disrupts free radicals by reacting with ROS directly or by reacting with LOOHs, terminating formation of free radicals. Vitamin E also has an anti-inflammatory effect; it interferes with the eicosanoid pathway, thus decreasing inflammation through inhibition of the prostaglandin pathway.¹⁵ The end result is a decrease in inflammation. As vitamin E can absorb a maximum of 295 nm of UVR, its photoprotective effects are probably the result of its antioxidative properties and not its absorption properties.

Many studies have demonstrated the potent antioxidative effects of ingested vitamin E,¹⁵ but few studies have been conducted on the antioxidative effects of topically applied vitamin E.¹⁶ Recently, Bissett et al¹⁷ studied the role of topical vitamin E in UVR-induced changes in the skin. They reported a free-radical-quenching, photoprotective effect on the skin. The efficacy of vitamin E may depend on its formulation and delivery mode. For instance, in one study, α -tocopherol sorbate, but not α -tocopherol or tocopherol acetate, significantly de-

creased the UVR-induced free-radical flux in the skin.¹⁵ Various formulations and concentrations of topical vitamin E are available. Concentrations range from 0.2% to 20% (benefits of higher concentrations have not been established). Studies have demonstrated that topical vitamin E protects against sunburn,¹⁵ protects against clinical signs of photoaging (ie, wrinkling, roughness, elastosis, lentiginos),¹⁸ prevents UVR immunosuppression,¹⁹ and may even accelerate wound healing.

Vitamin E is a potent, safe, and well-tolerated endogenous antioxidant with many beneficial effects on the skin, including prevention of sun-induced damage. However, clinical trials are needed to evaluate the benefit of topical vitamin E in prevention or reversal of sun damage and in combined use with other antioxidants.

SUMMARY

There is a significant lack of published data regarding topical use of antioxidants in prevention or reversal of UVR-induced aging of skin. Although results of studies have supported use of topical antioxidants, no studies have been done on combined use of antioxidants in the treatment of photoaged skin. Combinations of antioxidants should be more effective than individual agents alone, as many of these antioxidants have different mechanisms of action and different functions. For example, tretinoin affects collagen synthesis, and vitamin C is a required cofactor for collagen synthesis. Glycolic acid, an AHA, enhances glycosaminoglycan synthesis, thus improving the dermal matrix on which new collagen is made. Therefore, combining glycolic acid, tretinoin, and vitamin C seems natural. Vitamin C works in concert with the very potent vitamin E by regenerating oxidized vitamin E. If the science supports the hypothesis, combinations of different antioxidants can be formulated within a single product to provide the user with maximum antioxidative protection.

Because UVR generates highly reactive oxygen free radicals, all antioxidant therapies should be combined with sunscreens or sunblocks to maximize photoprotection and to prevent further sun damage.

Clinical studies are needed to evaluate combined use of topical antioxidants in prevention and reversal of histologic and clinical manifestations of photoaging.

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