

Review of Photodamage and Oxidative Stress and Protection Provided by Topical Antioxidants

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Abstract

Sunscreens are useful, but their protection is not enough because of inadequate use, incomplete spectral protection and irritation. New methods to protect skin from photodamage are necessary for the prevention of skin cancer and photoageing. Skin naturally uses antioxidants (AOs) to protect itself from photodamage. This scientific article summarises what is known about how photodamage occurs, why sunscreens – the current gold standard of photoprotection – are insufficient and how specific topical AOs help protect against skin cancer and photoageing changes. The antioxidants reviewed are those AOs for which sufficient information is available to document their potential topical uses and benefits. Their topical use may favourably supplement sunscreen protection and provide additional anticarcinogenic protection.

Keywords

Topical antioxidants, ultraviolet (UV) irradiation, skin cancer, reactive oxygen species (ROS), L-ascorbic acid, phloretin, ferulic acid, minimal erythral dose (MED), sunburn cells, p-53, thymine dimer, Langerhans cells, matrix metalloproteinase 9 (MMP-9), cosmeceuticals, non-invasive procedures

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Photodamage

Sunlight, coupled with living in an oxygen-rich atmosphere, causes unwanted and deleterious stresses on skin. The most severe consequence of photodamage is skin cancer. Less severe photoageing changes result in wrinkling, scaling, dryness and mottled pigment abnormalities consisting of hyper- and hypopigmentation.¹ Ultraviolet (UV) irradiation caused by sun exposure is a potent generator of oxidative stress in the skin and leads to acute inflammatory reactions such as erythema and sunburn and chronic reactions including premature skin ageing and skin tumours. Exposure of mammalian skin to UV 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 the sunburn reaction, as well as to photocarcinogenesis and photoageing.²

Reactive Oxygen Species

ROS are an inherent part of the anabolism and catabolism of tissues, including skin. Most oxygen in the body is used in cellular metabolism. Through a series of one-electron subtractions, molecular oxygen is, in sequence, changed to superoxide anion, hydrogen peroxide, hydroxyl radical and, finally, to water. Most reactions occur in mitochondria and are related to energy production. Cellular enzymes and controlled metabolic processes ordinarily keep oxidative damage to cells at a minimum.^{3–5} However, in times of increased oxidative stress, including high metabolic demands and outside forces such as sunlight, smoking and pollution, oxidative damage may occur.³ Free radicals, known to

cause the most damage, are defined as atoms or molecules with an unpaired electron. Examples include the superoxide anion, peroxy radical and hydroxyl radical.⁴ These molecules are extremely chemically reactive and short-lived; they react at the place where they are created. Other reactive molecules, such as molecular oxygen, singlet oxygen and hydrogen peroxide, are not free radicals, but are capable of initiating oxidative reactions and generating free-radical species. Together, these free radicals and reactive oxygen molecules are called ROS.^{4,5}

Photocarcinogenesis

UVB radiation is a complete carcinogen and can generate squamous cell carcinomas in mammals.⁶ DNA absorbs UVB, leading to signature UV-induced DNA mutations C3T and CC3TT. The UV action spectrum for generation of squamous cell carcinoma occurs mostly in the UVB range, although there is a peak of activity in the UVA range (320–400nm).⁷ Whereas UVB is important for tumour initiation, UVA predominantly causes tumour promotion.⁸ Compared with UVB, UVA generates more oxidative stress.^{9–12} At levels found in sunlight, UVA is 10 times more efficient than UVB at causing lipid peroxidation.¹³ UVA is more cytotoxic than UVB.⁸ UVA damages DNA by causing strand breaks and oxidation of nucleic acids.

Sunscreens Are Not Enough

One of the cornerstones of UV protection is the orthodox use of strong sunscreens. However, even if this is largely effective in blocking harmful

UV rays, the protection is not 100% successful because of uneven application, insufficient amount, rub-off with sweat, etc.¹⁴ Sunscreens are the 'gold standard' for protecting skin from photodamage. Many chemicals have been developed that absorb UV light efficiently and protect against erythema. However, in actual use, sunscreens provide much less protection than expected. Sun protection factor (SPF) is measured and tested at an application to skin of 2mg/cm². Controlled studies of actual sunscreen use reveal that sunscreens are applied to skin at only 0.5mg/cm² or less. SPF is not linearly proportional; thus, at an application of 0.5mg/cm² no sunscreen provides more than three- to five-fold protection.^{15,16} Moreover, important biological events such as DNA damage, as measured by thymine dimer and 8-hydroxy-2'-deoxyguanosine formation, as well as p53 induction and UV immunosuppression, continue at sub-erythral levels of irradiation.^{17,18} Sunscreens may give a false sense of security as none provides full spectral protection against UV light. Some of the ingredients contained in sunscreens also may become free radicals when activated by UV irradiation, and sunscreen chemicals may be absorbed into the skin to potentially cause harm.^{19,20}

Antioxidant Review

The skin is equipped with an elaborate system of antioxidant (AO) substances and enzymes to protect it from oxidant stress generated by sunlight and pollution. The endogenous AO capacity of the skin is a major determinant in its response to oxidative stress-mediated damage.¹ The normal ageing process and environmental stress can deplete the epidermis of protective AOs. Thus, AOs potentially constitute an important group of pharmacological agents capable of preventing the occurrence and reducing the severity of UV-induced skin damage and skin ageing.²¹ Low-molecular-weight, non-enzymic AOs include L-ascorbic acid in the fluid phase, glutathione in the cellular compartment, vitamin E in membranes and ubiquinol in mitochondria. Low-molecular-weight AOs work in tissues as a co-ordinated interactive group of chemicals related to chemical structure, position in the tissue and relative redox potential. Therefore, when an ROS is generated in a lipophilic structure and is reduced by alpha-tocopherol, the oxidised tocopherol can be regenerated by ubiquinol or L-ascorbic acid. In turn, dehydroascorbate can be reduced by glutathione, which, in turn, can be reduced by the nicotinamide adenine dinucleotide phosphate (NADP) pool. This balance may be essential for function and the system could potentially fail when any step in the process becomes rate limiting.²²

Topical Antioxidants

Low-molecular-weight AOs undergo depletion in the process of protecting skin against oxidative stress. Therefore, it is beneficial to add to the skin reservoir by topically applying AOs. Direct application has the added advantage of targeting the AOs to the area of skin needing protection. For topical application to be useful, however, several obstacles must be overcome: AOs are inherently unstable compounds, allowing them to function in redox reactions. Instability makes them difficult to formulate in an acceptable, stable composition for cosmetic use. To protect deeper layers of skin, AOs need to be formulated in a way that delivers them efficiently into skin. Concentrations need to be optimised to maximise skin levels.¹ Finally, AOs need to have photoprotective effects including reduction of erythema, reduction of sunburn cell formation, reduction of DNA changes such as thymine dimers or oxidised nucleotides, reduction of UV immunosuppression, reduction of pigment abnormalities and, eventually, reduction of skin cancer and photoageing changes. Below is a review of AO candidates for topical application.²¹

Vitamin C

Also known as L-ascorbic acid, vitamin C is a highly water-soluble, sugar-like, low-molecular-weight alpha-ketolactone. It is also the body's major aqueous phase reductant.²³ By a stepwise donation of an electron, the resulting ascorbate free radical that is formed is more stable than other free radicals and can serve as a free-radical scavenger. After loss of a second electron, the resulting oxidation product, dehydroascorbic acid, can be regenerated by dehydroascorbic acid reductase or, as frequently happens, may decay as the lactone ring irreversibly opens. In addition to its AO properties, L-ascorbic acid is essential for collagen biosynthesis; it serves as a co-factor for prolyl and lysyl hydroxylases, enzymes necessary for molecular stability and intermolecular cross-linking.²⁴ Additionally, it is important in the regulation of collagen synthesis and may inhibit elastin biosynthesis. Therefore, it could be useful for reducing the increased elastin accumulation that occurs in photoaged skin.²⁵ L-ascorbic acid reduces pigment synthesis in skin by inhibiting tyrosinase. L-ascorbic acid also improves epidermal barrier function, apparently by stimulating sphingolipid production.²⁶

Vitamin E

Vitamin E is the body's major lipid-phase AO. The major AO function of vitamin E (or alpha-tocopherol) is to prevent lipid peroxidation. When an ROS attacks membrane lipids, a peroxy radical may form that can create more peroxy radicals, resulting in a chain reaction that may threaten the structural integrity of the membrane.²⁷ Tocopherols and tocotrienols scavenge the peroxy radical, ending the chain reaction. Vitamin E may also quench singlet oxygen.^{27,28} Once oxidised, vitamin E can be regenerated back to its reduced form by L-ascorbic acid, allowing it to be reactivated without creating a new membrane structure.²⁸ Several studies have documented photoprotective effects when vitamin E was topically applied to mammal skin. Follow-up studies to investigate the mechanism of inhibition of photocarcinogenesis have revealed that alpha-tocopherol inhibited UV-induced cyclopyrimidine dimer formation in the epidermal p53 gene.²⁹ In addition to its photoprotective effects, alpha-tocopherol inhibits melanogenesis; it inhibited melanin formation in melanoma cells and demonstrated inhibitory activity against tyrosinase and tyrosine.³⁰ It should be noted that alpha-tocopherol has modest UV absorption near 290nm and that some of its topical photoprotective effects may be related.³¹

Plant Antioxidants

Plants also have to protect themselves from the sun. In fact, they have an even greater struggle to avoid being oxidised to death because they are unable to move to avoid sunlight. Virtually all plants synthesise vitamin C and vitamin E³² to protect themselves from the sun. In addition, they synthesise flavonoids, which are polyphenolic compounds that are powerful AOs.³³

Phloretin

This enzyme is found in both the flesh and peel of apples, and is classified as a special family of flavonoids called dihydrochalcones; its unique molecular structure is responsible for potent AO activity in peroxynitrite scavenging and in the inhibition of lipid peroxidation.²¹

Ferulic Acid

An abundant phenolic phytochemical found in plant cell wall components such as arabinoxylans as covalent side chains, ferulic acid is an organic compound used by all plants to protect themselves

Figure 1: Thymine Dimer Formation Is Significantly Reduced by Phloretin CF Compared with Vehicle

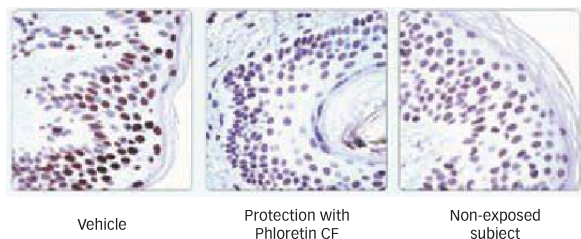


Figure 2: Results of a Once-a-day Application of SkinCeuticals Phloretin CF at Baseline (left) and at 24 Weeks (right)

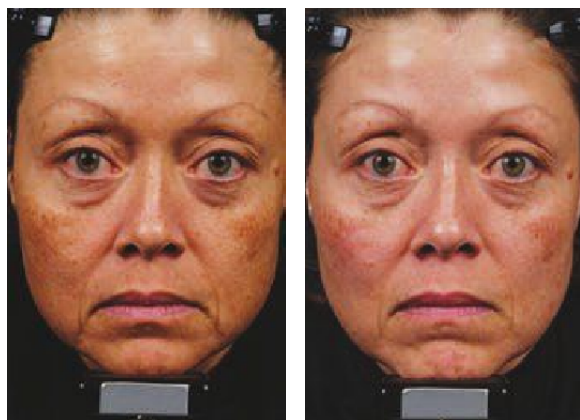


Figure 3: Topical Antioxidants and Sunscreens Are Complementary Against Oxidative Stress



from the sun. It is related to trans-cinnamic acid. Ferulic acid, like many phenols, is an AO that is reactive towards free radicals and is important in stabilising and empowering vitamin C. It is also able to absorb UVAII light.²¹

Combination Antioxidants Developed in Stepwise Fashion

In recent years, the protective effects of AOs applied topically to skin to prevent UV-induced oxidative damage have been investigated. Although there are many known low-molecular-weight AO substances, their efficacy is limited if they fail to penetrate skin.²¹

First Generation

A systematic study of endogenous AOs resulted first in a topical formulation of L-ascorbic acid that was maximised for chemical stability, concentration, availability and subsequent photoprotection for skin.³⁴

Second Generation

Addition of α -tocopherol improved stability and photoprotection, demonstrating the interacting balance achieved by combination AOs.³⁵

Third Generation

Investigation of plant phenolic AOs revealed that ferulic acid improved the stability of the AO formulation of L-ascorbic acid + α -tocopherol as well as its photoprotective properties.³⁶⁻³⁸

Fourth Generation

Subsequent studies have identified phloretin, another plant AO found in both the flesh and peel of apples, as a useful and potent AO capable of penetrating the skin and interacting with other AOs to provide effective photoprotection.²¹ Each new combination builds on previous science and formulations – precisely calibrating selected ingredients and maximising their concentrations to yield optimal results.

The study described below shows that the once-a-day application of a topical AO serum combining L-ascorbic acid, phloretin and ferulic acid:

- protects skin from oxidative damage complementary to sunscreens by working into the skin (extracts of the study published in *Journal of Cosmetic Dermatology*);²¹ and
- improves the visible aspects of photodamage, including fine lines, wrinkles, hyperpigmentation and loss of radiance or dull skin texture (extracts of the article published in *Dermatology Times*).¹⁴

Efficacy Study Using Biomarkers of Human Skin Damage Formulations²¹

The preparation used in this study was a solution of 10% L-ascorbic acid in a hydroglycolic base (water, butylene glycol, dipropylene glycol and ethanol) containing 0.5% ferulic acid and 2% phloretin: phloretin CF. The solution was adjusted to pH 2.5 to achieve maximum topical absorption. A vehicle-only solution (without the active ingredients) served as a control. These mixtures had no appreciable SPF value as measured by *in vitro* UV absorption profiles.

Subjects/Methods²¹

Ten subjects (18–60 years of age, Fitzpatrick skin types II and III) were randomised and treated with the AO formulation or vehicle control (two test sites) on the lower back for four consecutive days.

On day three, the minimal erythema dose (MED) was determined for each subject at a different site on the back. On day four, the two test sites received solar-simulated UV irradiation 1–5x MED and 1x MED intervals. On day five, digital images were taken and

4mm punch biopsies were collected from the two 5x MED test sites and a control site from each subject for morphology and immunohistochemical studies.

Results²¹

UV irradiation significantly increased the erythema of human skin in a linear manner from 1x to 5x MED. As early as 24 hours after exposure to 5x MED of UV radiation, there were significant increases in sunburn cell formation, thymine dimer formation (see *Figure 1*), matrix metalloproteinase-9 (MMP-9) expression and p53 protein expression. All these changes were attenuated by the AO composition. UV irradiation also suppressed the amount of CD1a-expressing Langerhans cells, indicating immunosuppressive effects of a single 5x MED dose of UV radiation. Pre-treatment of skin with the AO composition blocked this effect.

Conclusion²¹

This study confirms the protective role of a unique mixture of AOs containing vitamin C, ferulic acid and phloretin on human skin from the harmful effects of UV irradiation. In addition to being a potent AO, phloretin may stabilise and increase the skin availability of topically applied vitamin C and ferulic acid. We propose that AO mixture will complement and synergise with sunscreens in providing photoprotection for human skin.

Clinical Study

Methodology¹⁴

In the 24-week, multicentre, controlled clinical trial, 55 women 35–65 years of age with mild-to-moderate periorcular fine lines and coarse wrinkles and mild-to-moderate hyperpigmentation of the face and hands received a once-daily application of the product to the face, neck, chest and the dorsal aspect of both hands. Objective and subjective irritation parameters were evaluated, and Chroma Meter (Minolta) measurements of the same selected hyperpigmented lesion of the face – as well as Cutometer (CK Electronic) measurements of the skin's visco-elastic properties – were taken. Additionally, silicone replicas of the periorcular wrinkles were taken at each study visit, and digital images of the face were taken to evaluate efficacy. A clinician graded and assessed the improvements of the visible aspects of photodamaged skin at baseline and at weeks four, eight, 12, 18 and 24.

Results¹⁴

Efficacy

The results showed that statistically significant improvements could be seen throughout the study compared with baseline for all of the

clinical assessment parameters (see *Figure 2*). Results of the Chroma Meter analyses showed an increase in skin brightness/lightness at each follow-up assessment, and a reduction in skin pigmentation at week 18. The silicone replicas showed an improvement in skin texture. By week eight, more than 79% of participants were satisfied with the overall appearance of their skin, more than 77% felt that the topical product reduced the appearance of fine lines and wrinkles and more than 62% saw a reduction in brown spots and hyperpigmentation.

Tolerance

Although SkinCeuticals Phloretin CF contains high concentrations of vitamin C, phloretin and ferulic acid, the topical serum was well-tolerated by volunteers throughout the study (sponsored by L'Oréal USA), and none of the participants showed any significant increases in erythema, oedema, scaling/peeling, burning, itching or tingling.

Conclusion

Pollution, sunlight radiation and even the body's metabolism all contribute to oxidative stress in the skin. Information from various studies supports the notion that photocarcinogenic damage can be a result of overexposure to sunlight and its relationship to oxidative stress. AOs work together in skin, supporting and regenerating each other.

Topical AOs may provide several advantages for photoprotection not provided by dietary supplements. If AOs can be delivered into skin, they can be targeted to exposed skin, circumvent physiological barriers to systemic tissue delivery and accumulate in pharmacological concentrations. Their percutaneous absorption should supplement the natural AO protection present in skin and provide supplemental reserves as oxidative stress depletes AO stores. The topical use of AOs may favourably supplement external sunscreen protection by providing an additional internal shield (see *Figure 3*). ■



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