ELSEVIER

Topical Vitamin C in Aging

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Vitamin C (ascorbic acid) is essential for life in humans. Its deficiency causes scurvy. Indeed, the name "ascorbic" is derived from ascorbutic (*scorbutus* = scurvy). Scurvy was described in writings by the ancient Egyptians, Greeks, and Romans and during the Crusades in the 13th century. In the 18th century, Lind, a British naval surgeon, found that citrus fruits rapidly cured the disease common to travelers, especially seamen.[1] Today scurvy still exists, not only in Third World countries but also in industrialized nations, largely as a result of urban poverty. The groups found to have modern-day scurvy in this country are alcoholics and the institutionalized elderly.[2]

Since the discovery in the 1930s that vitamin C is the antiscorbutic factor, much research has been carried out elucidating vitamin C's metabolic role and its uses in pharmacologic doses. Pertinent to dermatology are its roles in collagen synthesis and as an antioxidant. Most of the supplementation research has considered ingested or parenteral vitamin C. A relatively small body of literature has focused on topical application for photoprotection and treatment of photoaging.

This review will highlight these data. By way of background, we shall also discuss the biochemistry and the physiologic and pharmacologic effects of vitamin C.

Structure and Biochemistry of Ascorbic Acid

Chemically, ascorbic acid (AA) is an alpha-ketolactone with the structure shown in Fig. 1. At physiologic pH, AA exists as the monovalent hydroxyl anion. This and its polar moieties restrict ascorbate to aqueous compartments. By a stepwise donation of two electrons, ascorbate is oxidized to dehydro-L-ascorbic acid (DHAA). The intermediate compound after donation of one electron is the ascorbate free radical. Fortunately, as this transient compound is more stable than other free radicals, ascorbate is an effective free radical scavenger. DHAA can be reduced to ascorbate; this reaction can be catalyzed by DHAA reductase, or it decays to 2,3-diketogulonic acid, a step in which the lactone ring irreversibly opens.

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Absorption and Excretion of AA

Primates, including humans, and guinea pigs, unlike other mammals, do not synthesize ascorbate from glucose owing to a deficiency of L-gulono-γ-lactone oxidase, the final enzyme in ascorbate synthesis. Vitamin C must then be ingested for survival. Plants, especially citrus fruits and dark-green leafy vegetables, are the richest sources. Intestinal absorption is 80–90% efficient with normal dietary intake.[3] Ascorbate is actively cotransported with sodium against an electrochemical gradient into the intestinal (small bowel) epithelial cell. Once it is inside, a concentration gradient is created by both brush border absorption and intracellular reduction of DHAA to ascorbate. Facilitated diffusion of ascorbate through the basolateral membrane is sodium independent and follows a concentration and electrochemical gradient.

Renal handling of ascorbate is by a similar mechanism, accounting for near-complete resorption of glomerular-filtered compound. Only in excess states are high levels of ascorbate and, significantly, one of its metabolites, oxalate, found in the urine. The latter is pertinent, as it accounts for one of the few potential clinical toxicities of systemic vitamin C supplementation, oxalic acid renal stones. Intestinal absorptive efficiency likewise declines with repleted or oversaturated states.

Biochemistry of AA

Role in Redox Reactions in General

AA's stepwise oxidation and subsequent regeneration make it an effective reducing agent (electron donor) biochemically. Besides its role as a free radical scavenger, AA functions in biologic systems as an enzymatic cofactor, providing electrons for ferric and cupric metalloions. Therefore, ascorbate's biochemical functions can be broken down broadly into its participation in enzymatic systems and its antioxidant role.

Cofactor functions of AA

Ascorbate serves as a cofactor in a number of enzyme systems.[4] In general, these enzymes are monooxygenases or dioxygenases utilizing reduced copper and iron metalloions, respectively. For some of the enzyme systems, the need for ascorbate is not absolute. Other reducing agents can serve to keep the metalloion cofac-

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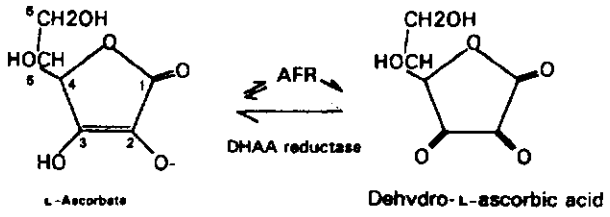

Figure 1. Chemical structure of ascorbic acid.

tors in their reduced forms. The enzymes' activities are most stimulated, however, in the presence of ascorbate. In the case of dioxygenases utilizing Fe^{2+} and ascorbate as cofactors, alpha-ketoglutarate and O_2 are consumed, and succinate and CO_2 are produced. Often the reaction product entails hydroxylation of the peptide substrate. The general reaction is shown below:

$$\text{Peptide substrate} + \alpha\text{-ketoglutarate} + O_2 \xrightarrow{\text{Ascorbate/Fe}^{2+}} \text{Product (substrate–OH)} + \text{succinate} + CO_2$$

Hydroxylation of procollagen is one such enzyme system in which ascorbate participates and is of particular importance in dermatology. Collagen is the most abundant animal protein and is uniquely synthesized. AA appears to influence production of collagen by posttranslational and transcriptional mechanisms. One of the best-known functions of AA is as a cofactor in the hydroxylation of lysine and proline residues of procollagen as it is being translated within ribosomes. Three dioxygenase enzymes catalyze these reactions: prolyl 3-hydroxylase, prolyl 4-hydroxylase, and lysyl hydroxylase. Formation of 4-hydroxyproline contributes to helical stability of the collagen molecule, while hydroxylation of lysine allows intermolecular cross-links to form, stabilizing the collagen fiber. The exact function of 3-hydroxyproline is unclear. In all three reactions, as with the several dioxygenase reactions in which ascorbate participates, ascorbate is consumed nonstoichiometrically. One explanation for this is that AA is not involved in the substrate/product cycle catalytically utilizing reduced iron.[5] Only when a nonhydroxylatable sequence is encountered does the enzyme-bound Fe^{2+} get "wasted" to Fe^{3+}, then requiring reduction by AA.

In recent years, ascorbate's role in influencing quantitative collagen synthesis in addition to stimulating qualitative changes to the collagen molecule has been proposed. One way ascorbate may stimulate collagen synthesis is by directly and specifically activating collagen gene regulation, both by increasing transcription rate and by stabilizing procollagen mRNA.[6,7] Another controversial mechanism is initiation of lipid peroxidation. Here, AA acts in a prooxidant capacity to effect membrane lipid free radical formation (peroxides). This increase in lipid peroxidation leads to an increase in malondialdehyde, a product of lipid peroxidation, which somehow stimulates collagen gene expression.[8] This mechanism has recently come into question, however, as it may occur as an artifact of in vitro systems.[9]

Other examples of enzymatic processes utilizing AA are carnitine biosynthesis (two ascorbate-associated hydroxylases), norepinephrine biosynthesis (AA serves with copper as a cofactor for a monooxygenase), activation of a number of peptide hormones by alpha-amidation (monooxygenase), and tyrosine catabolism (another Fe^{2+}-hydroxylase).[4]

Antioxidant Function of AA

OXIDATIVE STRESS Much of the recent research on vitamin C has focused on its role as a free radical scavenger. The term "free radical" as applied to aerobic organisms translates to oxygen free radicals. If one includes excited state oxygen species, such as singlet oxygen ($^1O_2$), which is not a free radical per se, one may more broadly apply the term "reactive oxygen species" (ROS).

Endogenous or physiologic production of ROS begins by the partial reduction of O_2 to the superoxide radical, $O_2^{\bullet-}$. Single electrons are generated from a variety of physiologic sources, including mitochondrial electron transport chains, soluble oxidase enzymes (e.g., NADPH, as in activated neutrophils), various quinones, and cytochrome P-450.[10] Approximately 1% of O_2 reduced results in production of $O_2^{\bullet-}$.[11] Superoxide dismutates to form hydrogen peroxide, H_2O_2. Hydrogen peroxide and $O_2^{\bullet-}$, in the presence of catalytic amounts of iron or copper metalloions, can generate the hydroxyl radical ($HO^{\bullet}$). As free radical species are products of these reactions, which can in turn propagate more reactions, a single event can initiate a free radical chain reaction.

Exogenous sources of ROS initiation include UV light, ozone, cigarette smoke, and dietary quinones and quinoid drugs.[10] Singlet oxygen is produced by absorption of incident light of particular wavelengths by excitable endogenous molecules. This energy is then transferred to an adjacent triplet (unexcited) oxygen molecule, raising O_2 to the singlet state. This ROS therefore exerts its effects primarily in the skin and the eyes. "Oxidative stress" is a term given to the sum total effects of ROS from these physiologic and exogenous sources.[12]

Oxidative damage occurs by a number of mechanisms.[13] DNA takes thousands of oxidative hits each day, many of them mutagenic. Structural or enzymatic proteins can be distorted or inactivated directly by oxidative damage. This has been causally related to cataract formation. Lipid peroxidation entails formation of peroxide radicals along the polyunsaturated fatty acid side chains of lipid. A single initiating event can lead to many cycles of peroxidation (another free radical chain

reaction), leading in turn to breakdown products that are themselves DNA or protein damaging. Lipid peroxidation has been linked to atherosclerosis through oxidative damage to low-density lipoprotein (LDL).[14]

ANTIOXIDANT PROTECTION Fortunately, elaborate systems to deal with oxidative stress have evolved to maintain cellular integrity.[11] Biologic antioxidants can be categorized as enzymatic (superoxide dismutase, catalase, and glutathione peroxidase and reductase) and nonenzymatic (ascorbate, tocopherol, beta-carotene, reduced coenzyme Q, or ubiquinol, and reduced glutathione). These antioxidant systems act as primary intracellular defenses against reactive oxygen species before their interaction with macromolecules. The enzymatic antioxidants serve mainly intracellularly, being absent or present in small amounts extracellularly, and they prevent free radical chain propagation by interrupting initiation. Nonenzymatic antioxidants are small molecules that act in both the intra- and the extracellular spaces, although they contribute proportionally more extracellularly. This is particularly important in the intravascular and connective-tissue spaces.

Nonenzymatic antioxidants can be divided into lipid- and water-soluble molecules. Tocopherol (vitamin E) and beta-carotene, a precursor of vitamin A, are the main lipid-soluble nonenzymatic antioxidants. They are mainly localized to cell membranes and LDL. Alpha-tocopherol, the most active form of vitamin E, breaks free radical chain reactions. Given its location, tocopherol inhibits already initiated lipid peroxidation, in addition to having several other antioxidant effects. Beta-carotene interacts primarily with singlet oxygen by "deexciting" it.

Ascorbate is the main water-soluble nonenzymatic antioxidant. AA interacts with a wide variety of free radicals intracellularly and forms a front-line extracellular defense against free radicals in plasma.[15] Besides soluble sulfhydryl groups, AA is the most efficient antioxidant in aqueous compartments.

Ascorbate has many properties of an ideal free radical scavenger.[3] First, it is available in many tissues in humans, and in adequate supply. Second, AA is compartmentalizable; that is, it can accumulate within cells or certain tissues. This lends itself to the properties of intestinal epithelial absorption and renal tubule resorption discussed above. Third, AA is recycled. Mechanisms exist to reduce DHAA and the ascorbate free radical; thus, dietary acquisition is not biologically expensive. Fourth, ascorbate is a versatile antioxidant, able to interact with superoxide and hydroxyl free radicals, in addition to singlet oxygen. Fifth, ascorbate toxicity is tolerable. Megadoses of vitamin C—100 to 200 times the recommended daily allowance—are generally well tolerated. Ascorbate free radical, with its relatively low redox potential, does not propagate free radical chain reactions. Evidence exists, however, that DHAA is toxic to pancreatic beta cells. Corneal epithelial cells exposed to increased levels of DHAA had a decreased metabolic capacity. So, although ascorbate itself appears to be nontoxic, its redox partner, or perhaps the absence of AA, may be deleterious.

One additional important antioxidant role ascorbate plays is in the regeneration of alpha-tocopherol (the most active form of vitamin E) from the tocopherol radical.[16,17] This occurs nonenzymatically, presumably at the aqueous/lipid membrane interface. This recycling effect has been specifically shown in skin.[18] Thus, AA not only directly protects membranes and LDL from ROS generated in the aqueous phase but also indirectly protects them by reduction of vitamin E radical.

Effects of Reactive Oxygen Species in the Skin

ROS no doubt produces oxidative damage in the skin and is reviewed in more detail in other articles in this issue. UV light, ozone, and other natural and synthetic environmental insults lead to cumulative damage, at least some of which is mediated by ROS.[14] ROS plays a role in skin carcinogenesis,[19–23] inflammation,[24–27] and photoaging.[28,29] UV light contributes directly to this damage, not only by generation of ROS but also by depression of antioxidant levels.[30]

Cutaneous inflammation can be induced by ROS[24] and, conversely, can induce formation of ROS.[25] Neutrophils are well known to produce superoxide radicals to aid in the killing of microorganisms. The respiratory burst of neutrophils further adds to ROS production. This added ROS burden can lead to autooxidative tissue damage at the sites of inflammation. This increased ROS burden may be implicated in a number of neutrophil-associated dermatoses, including cutaneous vasculitis, Behçet's disease, acne and rosacea, and psoriasis.[25] Likewise, the drugs used to treat these disorders have been found to have antioxidant effects.

Neutrophils themselves contain high intracytoplasmic concentrations of ascorbate, presumably to maintain cellular integrity during the respiratory burst.[26] Activated neutrophils greatly increase their intracellular ascorbate levels.[27] This increase was augmented by higher levels of extracellular levels of DHAA and eliminated by extracellular superoxide dismutase and catalase. This implies a preferential uptake of DHAA and rapid reduction back to ascorbate within neutrophils. Without extracellular oxidation of ascorbate to DHAA, neutrophils could not increase intracellular ascorbate.

ROS has long been suspected in cutaneous carcinogenesis, although the precise mechanism is just beginning to be elucidated. Ultraviolet B light has been shown to induce mutations in the tumor-suppressing gene p53, leading to formation of squamous cell carcinomas.[21] UVB was implicated by its "signature" C → T

and CC → TT dipyrimidine mutation sites. Moreover, in basal cell carcinomas these mutations occurred with high frequency at particular "hot spots" on the p53 gene.[22] This frequency was high enough to produce a second mutation on the other allele. Tandem double CC → TT mutations have been observed in an in vitro ROS-producing system.[23] It is interesting to note that this system did not rely on UV light, but instead utilized iron and copper metallions plus H_2O_2 to produce hydroxyl radicals, which then induced the mutations. The effect was inhibited by mannitol acting as an oxygen radical scavenger. Thus, ROS could be responsible for nonmelanoma skin cancers by inactivation of p53.

Systemic Vitamin C in the Skin

Recommended Allowances

As one of a few species required to ingest vitamin C, humans have asked the question, "How much is enough?" Dietary standards vary tremendously worldwide and have varied considerably in this country since the discovery that vitamin C is the antiscorbutic factor. As little as 10 mg daily is needed to prevent scurvy. As more is learned about the roles of ascorbate, however, many nutritionists question current guidelines.

The current recommended daily allowance (RDA) in the United States is 60 mg. This guideline is felt to be too low on the basis of a number of observations:

- Daily intake of 50 to 100 mg a day would be required to raise serum levels in humans to those in animals that synthesize ascorbate.
- Ascorbate turnover with saturated body stores is 60 mg a day. Just matching this amount by dietary intake does not take into account incomplete intestinal absorption.
- Vitamin C increases intestinal absorption of dietary iron. Iron deficiency continues to be a problem in many populations, including groups in "developed countries."
- Evidence exists for beneficial effects of supplemental vitamin C for wound healing, cancer prevention, cataract prevention, prevention of atherosclerosis, and enhancing immune mechanisms.

Though the RDA's intent is to replace daily turnover, many feel a recommended standard should take into account supplement data.[31]

Additional allowance is recommended for smokers and pregnant or lactacting women. For smokers, this is 140 mg/day. The exact mechanism of smoking's effect on levels of vitamin has not been elucidated, but it is presumed to be due to consumption of AA in inactivating oxidants from tobacco smoke. For pregnant women, high-dose supplementation is not recommended, as neonatal scurvy can occur postpartum (see below).

Plasma Levels of Vitamin C with Oral Supplementation

At steady state, the saturated body store of vitamin C is approximately 20 mg/kg of body weight. This corresponds to a plasma ascorbate level of 0.9 mg/dL.[32] The efficiency of intestinal absorption falls with increasing doses, with maximal absorptive capacity reached when 3 g is taken as a single dose. Three hours after this dose, plasma levels reach 3.5 mg/dL. Leukocyte levels, considered a more precise reflection of tissue levels, do not increase significantly with high-dose (up to 12 g) supplementation.

The half-life of ascorbate is 10 to 20 days and is dependent on plasma levels.[32] Saturated body stores allow approximately 4 weeks after cessation of vitamin C intake before signs of scurvy develop. Body stores appear to deplete faster after cessation of high-dose supplementation.[33] Rebound scurvy has been reported in those who abruptly stop supplementation.[34]

Cutaneous Levels of Vitamin C

Dermal and epidermal levels of vitamin C, as well as various other antioxidants, have recently been determined.[35] After separation of the epidermis and dermis by curettage of frozen specimens, homogenized samples were analyzed with high-performance liquid chromatography (HPLC). Epidermal ascorbate levels measured 3.8 μmol/g skin (669 mg/kg skin), more than five times the level in the dermis (0.72 μmol/g skin). This difference is much greater than that found in murine skin.[36] One may speculate that these different levels reflect different activities of antioxidants in the dermis and epidermis. The above measurements, however, are presumably per gram of *epidermis* or *dermis*. On a per-weight basis, epidermis would in vivo cover a considerably greater surface area than the same amount of dermis.

The response of cutaneous levels of vitamin C to oral supplementation is not known; however, these measurements in normal skin help establish a baseline from which differences with oxidative stress, aging, and cutaneous pathologies can be compared.

The effect of aging on antioxidant capacity has been studied in murine skin.[37] One aspect of the free radical theory of aging proposes a decrease in antioxidant capacity in older tissue. Although it neither disproves nor supports the theory, this decrease does not appear to happen. In old versus young murine skin, no differences were seen in any enzymatic and nonenzymatic antioxidants measured except glutathione peroxidase, which was decreased in older skin relative to younger skin.

Ultraviolet light's effect on skin levels of antioxidant has also been examined.[36] Exposure to UV generally depresses levels of cutaneous antioxidants, supporting UV light's role as an ROS generator and the skin's antioxidant system to counteract the oxidative stress.

In light of the above discussion of inflammation and ROS, levels of antioxidants have been seen to be depressed in cutaneous inflammatory states. Lesional skin in psoriasis has been shown to have decreased levels of superoxide dismutase.[38] This is in accordance with the observation that activation of neutrophils, and the subsequent increased production of superoxide radicals, leads to a stoichiometric consumption of ascorbate.[39] A corollary of this is the decrease in ascorbate levels within circulating leukocytes seen in systemic inflammatory diseases—for example, rheumatoid arthritis.[40]

Dermatologic Benefits of Ingested Vitamin C

WOUND HEALING Given ascorbate's function in collagen synthesis as well as the fact that wound healing in malnutrition in general and vitamin C deficiency in particular is prolonged and suboptimal, it stands to reason that vitamin C supplementation offers benefit in the healing of wounds. Wound healing appears to be improved by adequate vitamin C intake in the elderly[41] and oral supplementation for patients with pressure sores.[42] Postsurgical wounds heal abnormally and dehisce more often with low circulating ascorbate levels.[43]

CUTANEOUS AGING Aged skin, whether in culture or in vivo, has decreased baseline production of collagen when compared to younger skin.[44] Also, in cultures of dermal fibroblasts from elderly donors, proliferative capacity in the absence of ascorbate is lost.[45] When ascorbate is added to fibroblast cultures, elderly donor cells, previously on a plateau of growth, are stimulated to proliferate. In addition, collagen synthesis increases in similar proportions to increases in newborn donor cells. The molar concentration of ascorbate in these cultures was at least twice the average plasma levels found in healthy elderly adults (age 64 to 74) not taking vitamin C supplements. The implications are that supplemental vitamin C may slow cutaneous aging and may improve wound healing.

PREVENTION OF SKIN CANCER In animal models, dietary ascorbic acid has been shown to delay the incidence of cutaneous neoplasms induced by UV light.[46] To date, no epidemiologic or interventional data are available on humans.

Topical Vitamin C

Challenge of Delivery

The use of topically applied vitamin C is not a new concept. The cosmetic industry, capitalizing on the long-known effects of ascorbate on collagen, has undertaken to produce stable ascorbate products that can penetrate the skin, delivering L-ascorbate to the epidermis and dermis.[47] Ascorbyl esters, namely ascorbyl palmitate and phosphate, have in the past been marketed in cosmetics for the treatment of hyperpigmentation. The latter product has the commercial advantage of aqueous solubility, permitting a wide variety of cosmetic product formulations. It is also stable for at least 6 months and hydrolyzed to L-ascorbate by phosphatases present in skin. Palmitic esters of ascorbate are amphipathic molecules, having a polar ascorbate head and a long lipophilic tail. Ascorbyl palmitate is touted as being easily compounded in water creams, lotions, and oils. Also, ascorbyl palmitate is pH neutral, making it nonirritating when applied to the skin.[48] The activity of lipophilic esters of ascorbate is critically tied to the position of esterification. Positioning of the lipid moiety at the 2 or 3 position (see structure diagram) would not allow electron donation by ascorbate.

Lipid solubility would seem to be an advantage for a compound to penetrate the stratum corneum. Although aqueous and lipid-soluble forms of ascorbate have not been compared for their penetration into normal human skin, ascorbyl palmitate more effectively inhibited phorbol ester–induced ornithine decarboxylase activity, epidermal DNA synthesis, and tumor promotion in murine skin.[49] The authors point out that similar amphipathic compounds (eg, sorbitan monopalmitate, isoascorbyl palmitate), themselves not antioxidants, also help to inhibit the tumor promotion and markers of tumor promotion. Therefore, the enhanced antipromoter activity may not be due to ascorbyl palmitate's antioxidant effect.

A stable solution of aqueous ascorbic acid was elusive until fairly recently.[50] Previously, ascorbic acid, being a good reductant, rapidly became oxidized in aqueous solutions. This preparation is stable at a pH below ascorbate's first pKa. Thus, the molecule is nonionic and less lipophobic when applied—conditions essential for percutaneous absorption.

Pharmacologic Effects of Topical Vitamin C

SKIN LEVELS OF VITAMIN C APPLIED TOPICALLY Levels of L-ascorbate after topical application are known in animal models for the phosphate ester ascorbyl palmitate and aqueous L–ascorbic acid.[51,52] Human skin, obtained from autopsy, was also assayed for L–ascorbic acid and ascorbyl palmitate, in addition to alpha-tocopherol and its esters.[52] At 24 hours, penetration of L-ascorbate in human skin was 15% that of ascorbyl palmitate, 29% that of alpha-tocopherol, and even less than that of the tocopherol esters. As will be discussed below, however, these levels of ascorbate relative to other antioxidants do not correlate with antioxidant activity.

In porcine skin, measured by HPLC skin levels of ascorbate after topical application of a 10% aqueous solution.[51] Ascorbic acid–treated skin contained more than 25 times the amount of ascorbate found in vehicle-treated areas. Although not specifically tested, these levels are assumed to be much higher than those obtainable with oral supplementation. Additionally, as

seen with natural ascorbate levels, topical ascorbate–treated skin exposed to UV light experienced a 66% reduction in ascorbate levels from those in topically treated nonirradiated skin.

UV PHOTOPROTECTION Few human skin data are published showing the UV photoprotective effect of topically applied vitamin C. In a study of 10 healthy volunteers, post-UVB irradiation erythema was reduced in those pretreated with topical vitamin C.[53]

In the porcine model, 10% aqueous vitamin C was applied several times to the animals' skin, which was then irradiated with 400 mJ/cm^2 UVB and sampled 24 hours later.[51] Sunburn cell numbers were determined histologically and were seen to be significantly reduced in treated versus nontreated skin. UVB-induced erythema was measured by laser Doppler velocimetry 24 hours after a 2- to 3-MED dose of UVB had been applied to intact skin. This was likewise significantly reduced in vitamin C–treated skin. Finally, the effect of PUVA was tested with topically applied 8-methoxypsoralen with a UVA dose of 500 mJ/cm^2. Once again, after 48 hours, topical vitamin C–treated skin had less than half the number of sunburn cells per sample of untreated skin.

In a separate study, topical vitamin C in combination with a UVB sunscreen (PABA) on porcine skin produced a significant reduction in sunburn cell numbers. This was enhanced further with the addition of vitamin E to the solution. In PUVA-exposed skin, the effect of vitamin C was slightly enhanced by vitamin E and comparable to the UVA/UVB (oxybenzone) screen used; however, the combination of both antioxidants and oxybenzone produced a more than additive effect, nearly completely protecting the skin from phototoxicity.[54]

In the hairless mouse model, chronic skin damage from UVB and UVA was studied.[52] A 5% solution of ascorbate (prepared immediately beforehand) was applied 2 hours before exposure (three times weekly for UVB and five times weekly for UVA). It should be noted that the pH of the solution was 6–7; thus, ascorbate was applied as the monovalent anion, limiting percutaneous absorption. Visible physical and histologic parameters for photoaging in this animal had already been established.[28] Both topical ascorbate and alpha-tocopherol reduced UVB-induced skin wrinkling and delayed onset of skin tumors to a similar degree. Alpha-tocopherol had the advantage of compound stability, as ascorbate was seen to lose its effectiveness if the solution was not freshly prepared. The UVB photoprotection seemed to be dose related, reaching a maximum in this animal model at 5%. The UVB-induced photoprotection by topical L-ascorbic acid and alpha-tocopherol occurred despite lower levels obtained in the skin compared to these antioxidants' lipophilic esters. UVA-induced skin sagging was not prevented by topical antioxidants. It is interesting to note that mice given oral alpha-tocopherol, ascorbic acid, or beta-carotene experienced no reduction in photodamage.

RADIATION DAMAGE In a double-blinded, prospective, randomized, placebo-controlled trial, aqueous topical vitamin C (10%) was applied to the scalp of patients undergoing external beam radiation therapy for intracranial tumors to evaluate the radioprotection of this antioxidant.[55] No measurable reduction in radiation dermatitis was seen between skin treated with ascorbate and skin treated with vehicle alone.

ANTI-INFLAMMATORY EFFECTS Very few data exist for the use of topical vitamin C for anti-inflammatory effects. Perricone studied 12 patients with psoriasis in an unblinded fashion, comparing the effect of ascorbyl palmitate and vehicle with that of vehicle alone on symmetric psoriatic plaques. Erythema, scale, and thickness of the plaques treated with the ascorbate ester improved, while only scale improved in the vehicle-only treated lesions.[48] Neutrophil-derived ROS is believed to be involved in the pathogenesis of a number of inflammatory skin disorders, including cutaneous vasculitis, erythema multiforme, and Behçet's disease.[56]

Memon et al studied the use of topical chelating agents and topical antioxidants for inhibiting nickel-induced hypersensitivity.[57] Topical vitamin C was found to be ineffective, while the chelating agents had some benefit.

PROTECTION OF UV IMMUNOSUPPRESSION Topical application of L-ascorbic acid prevented UVB induction of tolerance in contact hypersensitivity.[58] This indirectly supports the role of ROS generated by UV exposure of the skin in inducing phototolerance.

Treatment of Photoaging

Despite an abundance of evidence that ROS induces both acute and chronic damage to the skin and that vitamin C helps reduce both endogenously and exogenously produced ROS, as well as stimulating collagen synthesis, no clinical trials have been carried out evaluating what would seem an obvious role for topical vitamin C—the treatment of photoaging. Topical ascorbic acid phosphate has been used with some success in the treatment of disorders of hyperpigmentation unrelated to photodamage.[47]

Conclusions

On the basis of a solid foundation of in vitro and animal model data, it can be stated that antioxidants, especially vitamins C and E, alleviate oxidative stress on the skin. Vitamin C has the additional advantages of replenishing vitamin E and stimulating dermal fibroblasts to synthesize collagen, a major target in chronic photoaging.

Cutaneous levels not obtainable by ingestion of vitamin C can be reached with topical application. Animal studies have shown significant acute photoprotective and chronic photoaging preventive effects from topical application. On the basis of circumstantial data, topical vitamin C should have a beneficial effect in the treatment of photoaging.

Given the low toxicity of ingested vitamin C, the barriers to study of topical vitamin C in human skin should be very low. These experiments need not be limited to photoprotection and photoaging prevention and treatment. Anti-inflammatory effects give reason to believe that vitamin C could be useful in treatment of inflammatory dermatoses, autoimmune disorders, and photosensitive diseases. With the more stable preparations of L-ascorbic acid now available, the way is paved to carry out substantive studies in humans.

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