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Topical Nutritional Antioxidants

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INTRODUCTION

In recent years, more and more cosmetic products have been formulated with antioxidants. These new products claim to "moisturize," "protect," and "rejuvenate" the skin.

The skin naturally uses nutritional antioxidants to protect itself from free-radical damage. Indeed, many antioxidants—most prominently vitamins C and E, the trace mineral selenium (Se), the soy extract genistein and ubiquinone—have been proven effective in protecting against ultraviolet (UV) damage to the skin and in actually reversing the appearance of aging by decreasing solar hyperpigmentation and small wrinkles when applied to the skin. Also, α-lipoic acid and ubiquinone may retard and reverse intrinsic as well as photoaging. Topical application of these antioxidants can give far higher concentrations in the skin than even maximal oral intake. However, the correct formulation is of utmost importance to attain eficacy. The challenge is to use the *correct form* of the antioxidant molecule, to keep the antioxidant *active* to attain a reasonable shelf-life for the product, and to achieve effective *transcutaneous* absorption that delivers effectively high concentrations of the active antioxidant to the dermis as well as the epidermis.

VITAMIN C

Background

Vitamin C (L-ascorbic acid) is the body's major aqueous phase antioxidant and is absolutely vital for life. All animals make their own vitamin C, except for humans and other primates, one species of Indian fruit-eating bat, and the guinea pig. In fact, a 130-pound goat synthesizes 13 grams of vitamin C per day, almost 200 times the American Food and Drug Administration (FDA) requirement (1). Not only do other animals make hundreds of times the vitamin C we ingest, but also, when under stress, they can make more than ten times their normal amount of vitamin C, a capability that we humans do not have (1).

Our skin is the organ that suffers most from environmental free-radical stress from exposure to sunlight, cigarette smoke, and other pollution. Furthermore, this contact actually depletes the level of vitamin C in skin. Even minimal UV exposure of 1.6 minimal erythema dose (MED) decreases the level of epidermal vitamin C to 70% of the normal level, and exposure to 10 MED decreases the vitamin C to only 54% (2). Exposure to 10 parts per million of ozone in city pollution decreases the level of epidermal vitamin C by 55% (3).

Mechanisms of Action

Vitamin C is itself not a sunscreen. Topical vitamin C protects against solar damage primarily as an antioxidant which deactivates the UV-induced free radicals, most significantly the superoxide anion, singlet oxygen, and the hydroxyl radical. Vitamin C is equally effective in protecting against both UVB (290–320 nm) and UVA (620–400 nm) (4). On both porcine and human skin, applying vitamin C decreases the acute erythema and sunburn suffered even when applied after sun exposure (4). Protection is confirmed by histologic examination. Treatment of porcine skin in vivo with topical 10% vitamin C decreases the number of abnormal apoptotic "sunburn cells" by 40% to 60% (4) and reduces the UV damage to DNA by 62% (4).

Topical vitamin C further prevents UV-induced immonosuppression (5). In approximately one-third of humans, the activity of the immune system is inhibited after exposure to sunlight. This immunosuppression is measured by the class of contact hypersensitivity to sensitizers such as poison ivy. Sunscreens only partially aid in the prevention of UV immunosuppression. Animal studies demonstrate that topical vitamin C prevents this UV-induced loss of contact hypersensitivity as well as UVB-induced tolerance.

Topical vitamin C is also directly anti-inflammatory (further accounting for decreased erythema after sun exposure). Laser resurfacing causes redness for at least three to four months. With vitamin C applied before and after laser resurfacing surgery, redness is decreased after only two months (6). Dermatologic surgeons recommend using topical vitamin C as long as possible prior to laser resurfacing and beginning again as early as fourteen days following surgery. Topical vitamin C can also be used effectively to treat the inflammation of rosacea (7).

The main action of vitamin C on the skin is direct stimulation of collagen synthesis. Vitamin C is an essential cofactor for the two enzymes required for collagen synthesis, prolyl hydroxylase (which makes the collagen molecule stable) and lysyl hydroxylase (which cross-links the collagen to give structural strength) (8). Recent research has further demonstrated that vitamin C acts directly on DNA to increase the transcription rate and to stabilize the pro-collagen messenger RNA, thus regulating and maintaining the intercellular amount of collagen (9).

Exciting experiments have demonstrated that vitamin C also has anti-aging effects. Studies in vitro compared newborn with elderly (80–95 year-old) fibroblasts (10). Elderly cells proliferate in vitro at only one-fifth of the rate of newborn cells. However, when vitamin C is added to the culture medium, the elderly cells actually proliferate better than normal newborn fibroblasts. Even the newborn fibroblasts proliferate almost four times better when exposed to vitamin C (10).

Not only do fibroblasts increase proliferation in the presence of vitamin C, but they also synthesize more collagen. Newborn fbroblasts synthesize a larger percentage of collagen than elderly cells, but again, when elderly cells are exposed to vitamin C in vitro,

they produce more collagen than the normal, newborn fibroblasts (10). Surprisingly, also the newborn cells double the amount of collagen synthesized (10).

Vitamin C further reverses the adverse appearance of photoaging by inhibiting tyrosinase (11), thereby fading unattractive solar lentigos. Because L-ascorbic acid may inhibit elastin biosynthesis (12), it may reduce the solar elastosis of photoaged skin.

Another important action of vitamin C on the skin is that topical vitamin C actually increases the synthesis of several very specific lipids of the skin surface (13). Not only does this mean that vitamin C helps the natural moisturization of the skin, but it also enhances the protective barrier function of the skin (14).

Challenges in Formulation

To optimize percutaneous absorption and full activity of vitamin C, the precise formulation is of utmost importance (15). Fortunately, the skin level of vitamin C can be increased significantly by topical application. Topical absorption was proven by radioactive-labeling studies in pigs. After treatment with 10% vitamin C cream, 8.2% was found in the dermis, and 0.7% was in the blood (4). Formulations containing 5%, 10%, 15%, 20%, or 25% vitamin C were tested: after 24 hours, 20% resulted in the highest skin levels, with maximized concentration in the skin after three days (16). Indeed, the level of vitamin C in the skin attained by topical application was over 27 times the level attained by high oral intake (16).

Since L-ascorbic acid is an inherently unstable molecule—making it an excellent antioxidant—creation of an effective topical delivery system is crucial. Many products contain stable derivatives which are not metabolized by the skin (such as ascorbyl-6-palmitate or magnesium ascorbyl phosphate) and therefore have no activity (16). Other formulations do not result in measurable absorption of vitamin C because they are not at the correct pH. Delivery of L-ascorbic acid depends upon removing the ionic charge achieved optimally at a pH of 3.5 (16). Having the pH below the pKa of ascorbic acid (pHa = 4.2) gives optimal activity as an antioxidant.

Substantiation of Efficacy

As cited above in the presentation of "Mechanisms of Action," the effcacy of topical vitamin C in neutralizing reactive oxygen species (ROS), protecting against both UVA and UVB damage, stimulating collagen synthesis, preventing UV immunosuppression, alleviating infammation, decreasing UV-induced pigmentation, and enhancing surface moisturization and skin barrier function has been repeatedly documented in controlled experiments.

Clinically, daily application of topical vitamin C 15% can partially reverse the appearance of photoaged skin. Improvement can be noted in as little as two to four months with optimal correction after at least four to six months. As shown in Figures 1 and 2, small wrinkles decrease and solar lentigines fade. Thus vitamin C not only prevents but also reverses much of the damage induced by UV.

VITAMIN E

Background

Like vitamin C, vitamin E is an essential nutrient, not synthesized by humans and supplied only by oral intake. The main natural sources are fresh vegetables, vegetable oils, cereals,

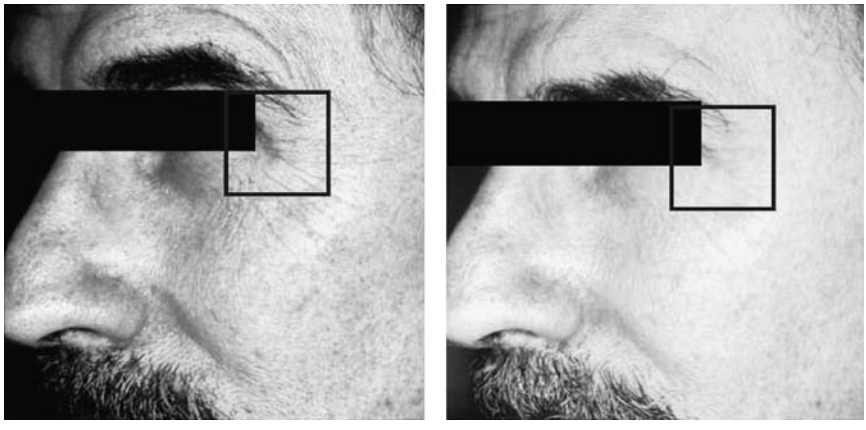


Figure 1 Decrease in small periorbital rhytides after daily application of vitamin C serum 15% (SkinCeuticals) for one year. *Source*: Photo courtesy of SkinCeuticals, Dallas, Texas, U.S.A.

and nuts. Natural vitamin E is the most important lipid-soluble, membrane-bound antioxidant in the body. Vitamin E is especially abundant in stratum corneum, delivered there by sebum (17,18). Its concentration is highest at the lower levels of the stratum corneum with a decreasing gradient outward. As the outermost defense of the body, the stratum corneum is first to absorb the oxidative stress of sunlight and pollution. Vitamin E is depleted in the process, so topical application can be particularly advantageous, especially since the lipophilic structure makes it cosmetically attractive for application and absorption.

Mechanisms of Action

The redox and free radical chemistry of vitamin E are well-documented (19). The major antioxidant role is the arrest of chain propagation by scavenging lipid peroxyl radicals. One molecule of tocopherol has the ability to scavenge two peroxyl radical molecules (20).

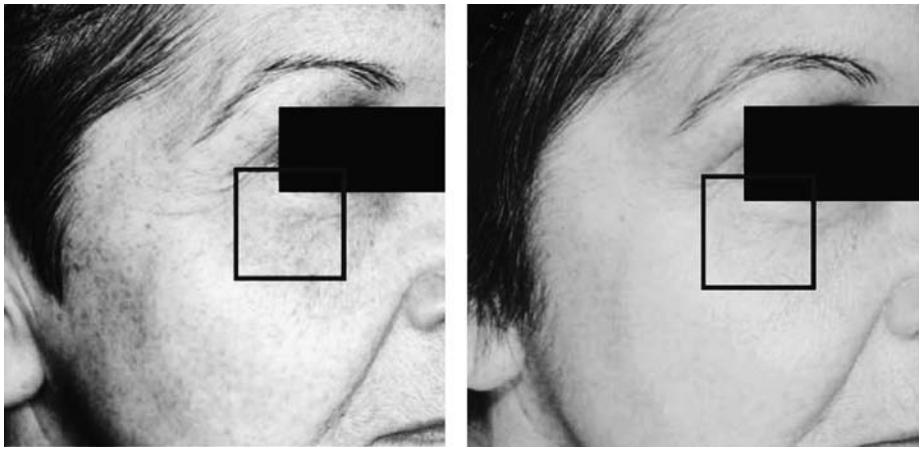


Figure 2 Lightening of UV-induced lentigines and hyperpigmentation after daily application of vitamin C serum 15% (SkinCeuticals) for one year. *Source*: Photo courtesy of SkinCeuticals, Dallas, Texas, U.S.A.

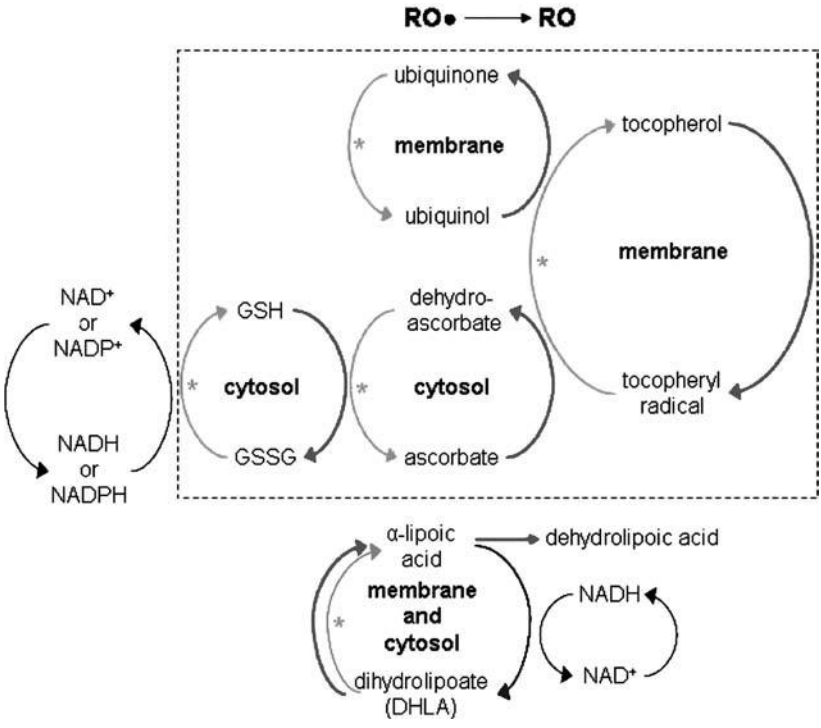


Figure 3 Interactions of low molecular weight antioxidants. The reactions which directly quench oxygen free radicals (RO•) are indicated by the dark gray arrows (RO• → RO); the reactions regenerating these antioxidants are also indicated by the light gray arrows. Reactions with arrows touching are directly linked. RO• generated in a cell membrane is reduced by tocopherol, forming a tocopheryl free radical which can in turn be quenched within the membrane by ubiquinol or at the membrane-cytosol junction by ascorbate (vitamin C). RO• generated in cytosol is directly reduced by ascorbate. The oxidized dehydroascorbate is reconverted to ascorbate by glutathione (GSH). Both α-lipoic acid and dihydrolipoic acid (DHLA) directly reduce oxygen free radicals. Also DHLA is itself a potent reducing agent which regenerates the oxidized forms of vitamin C, vitamin E, and oxidized glutathione (GSSH); this linkage is indicated by an asterix. *Source*: Adapted from Refs. 21, 22.

As shown in Figure 3, several hydrophilic coantioxidants, such as ascorbate and glutathione, regenerate vitamin E from the tocopheryl radical and thereby enhance the antioxidant capacity of vitamin E (21–23). Also, ubiquinol (coenzyme Q10) protects α-tocopherol from photo-oxidation by recycling (24).

There is extensive scientific evidence from animal studies that vitamin E is photoprotective. Topical vitamin E [even the metabolically less potent racemic or ester forms (see "Challenges in Formulation" below)] significantly reduces acute erythema, edema, and sunburn (25–30) if applied prior to UV exposure or (in some studies) immediately after. This has been confirmed histologically by decreased "sunburn cells" (27) and by electron microscopy showing epidermal cell repair and anti-inflammatory effects (28). Less DNA photodamage after UV with concomitant decreased p53 expression has been observed (29). Topical all-*rac*-α-tocopheryl acetate applied before UV exposure protected the hairless mouse epidermis against decreased DNA-thymidine incorporation and lipid peroxidation; given orally, this protected only against lipid peroxidation (30). This protection results from antioxidant (31) and/or anti-inflammatory activity (32,33).

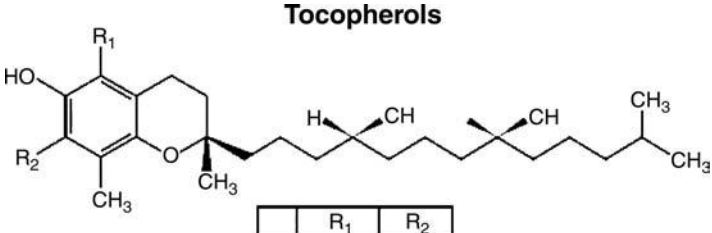
UV radiation directly alters DNA and induces free redicals (34,35) and epidermal lipid peroxidation (36), thereby initiating and promoting skin cancer (37). Vitamin E protects the skin from this chronic damage by: (i) quenching free radicals [as confirmed in vitro by protection by reducing radiation-induced lipid peroxidation (38)], and (ii) protecting specific membrane proteins containing Se or sulfur (39). Indeed, all-*rac*-α-tocopherol has been shown to prevent epidermal chemical carcinogenesis (40–42) as well as UV-induced photocarcinogenesis (43–46).

In hairless mice both oral (43) and topical all-*rac*-α-tocopherol combined with ascorbic acid (44) increased the latency period and decreased the number of UV-induced tumors. In Skh:2 hairless mice, both topical d-α-tocopherol (5%) and d-α-tocopheryl succinate (5%) as well as oral d-α-tocopheryl acetate signifcantly retarded the onset and decreased the incidence of UV-induced skin tumors; the topical succinate was less effective than the other two forms (25).

Challenges in Formulation

Several forms of vitamin E exist in natural dietary sources. The form which is found in mammalian tissues and has by far the greatest biologic activity is pure, nonesterified d-α-RRR-tocopherol (47,48) which has three methyl groups on the 6-chromal ring (Fig. 4). Humans use predominantly α-tocopherol because a specific α-tocopherol transfer protein selectively transfers α-tocopherol into lipoproteins (49). The other natural forms are beta, gamma, and delta which contain only one or two methyl groups on the 6-chromal ring. Relative to the alpha form, the beta, gamma, and delta RRR-tocopherols give only 42%, 72%, and 40%, respectively, of the protection against post-UV edema (50). The synthetic form is "dl" or "all-*rac,*" a mixture of eight stereoisomers. Not only is the decreased activity of the all-*rac* mixture of vitamin E important (51), but also the mixed all-*rac* form of vitamin E has been reported to cause allergic contact dermatitis (52) and erythema multiforme (53) when applied topically. No such adverse reactions have been reported with pure d-α-tocopherol.

Instead of the pure d-α-tocopherol, the synthetic isomers are esterified (to acetates and succinates) for use in commercial vitamins and some topical formulations because the esters are far more stable. The ester vitamin E acetate has been shown to be absorbed into the skin (54–56). This ester must be hydrolyzed to the active free tocopherol form before there is any biologic activity, a reaction which readily occurs in the stomach after oral ingestion or in cell and organ culture, but there is conflicting evidence as to what extent



	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Figure 4 Molecular structures of tocopherols.

this conversion occurs, especially in the stratum corneum (57–59). Thus the antioxidant potential of esterified vitamin E is far less than the natural tocopherol form (60). There is greater bioconversion in the lower nucleated epidermal cells (58,59) depending on the formulation (61). UVB exposure may enhance this conversion (62).

Stabilization of the non-esterified d-α-tocopherol to give a product an effective long shelf-life is a challenge in formulation. The stability can be enhanced by packaging in dark, sealed ampules for one application-only delivery, by formulating within liposomes, or by stabilizing chemically, often using other antioxidants. (Patents are pending for the latter two methods).

Substantiation of Efficacy

The scientific evidence of the benefcial role of vitamin E in protection from UV damage was discussed in detail above. Vitamin E has several other possible therapeutic roles in dermatology. Many anecdotal reports support the use of topical vitamin E to enhance wound-healing and to prevent hypertrophic scars; however, the benefits are controversial. Two controlled studies failed to show scar prevention by topical vitamin E (63,64). The stability and formulation of the topical vitamin E used may have effected these inconclusive studies. New research on diabetic mouse models suggests involvement of oxidative stress in diabetic wound healing showed significantly improved wound healing with topical vitamin E (65,66). Vitamin E may have a role in treating atopic dermatitis. Forty-three patients treated with oral vitamin E for eight months showed improvement and near-remission concomitant with a 62% decrease in serum IgE levels (67).

Furthermore, very exciting recent evidence suggests that oxidative stress is involved in the pathophysiology of melanoma and nonmelanoma cancer (68) and that vitamin E slows melanoma growth by promoting tumor cell apoptosis and inhibiting vascular endothelial growth factor-mediated angiogenesis (69,70).

Of great interest to the cosmeceutical formulations, there is the clinical evidence that topical vitamin E is indeed effective in reversing the appearance of photoaging. Figure 5 demonstrates the dramatic correction of periorbital wrinkles after four months of once-daily application of 5% d-α-tocopherol cream. Histologic confirmation of correction of the UV-induced epidermal hypertrophy with thickened stratum corneum, increased apoptotic "sunburn cells" in the basal layer, and disruption of dermal collagen and elastin was demonstrated in mice after eight weeks of topical treatment (KE Burke, L Ricotti, EG Gross, unpublished observation). Resolution of post-UV inflammation was also observed. Further electron microscopic analysis confirmed correction of collagen and elastin fiber

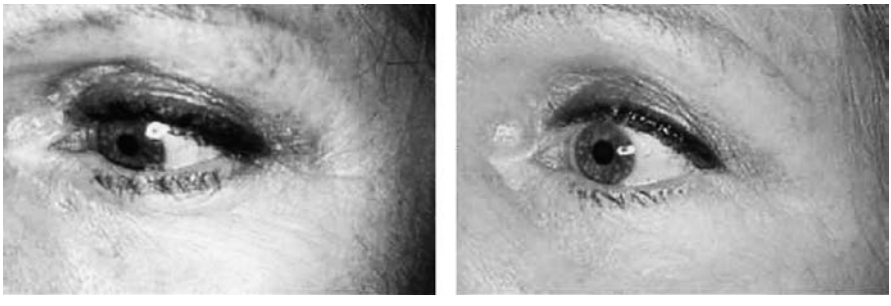


Figure 5 Correction of periorbital wrinkles after four months of once-daily treatment with 5% d-α-tocopherol cream.

damage and demonstrated repair of UV-induced disruption of collagen fibers and basement membrane anchoring fibrils. This correction of UV damage by topical d-α-tocopherol (5%) is as effective as that of topical tretinoin (retinoic acid), the "gold standard" of topical anti-aging.

SELENIUM

Background

Selenium (Se) was recognized to be an essential trace element in humans and animals in the late 1950s. A decade later, anticarcinogenesis was suggested by statistical correlation of decreased cancer mortality with increased Se in the diet in the United States (71). Scientific evidence indicates that indeed Se plays a role in cancer prevention (72–76). Se was shown to inhibit growth and to stimulate programmed cell death in a variety of cell culture studies, including human tumor cell lines in vitro (77). Hundreds of animal studies demonstrate that Se can reduce tumor yields: moderate Se supplementation at levels above the dietary requirements has been shown to decrease the number of tumors induced by several chemical carcinogens and viruses and to reduce the incidence of spontaneous mammary tumors (78) as well as the growth of other transplanted tumors (78).

Some, but not all, epidemiological studies have found a reduced risk for several kinds of cancer associated with a higher blood concentration of Se (79,80). A decreased Se concentration and glutathionine peroxidase (GPX) activity in blood and, interestingly, an increase of these parameters in malignant tissue was found in lung cancer patients (80). An initial study of 240 non-melanoma skin cancer patients in good general health demonstrated a significantly lower mean plasma Se concentration than control subjects without skin cancer (81). In fact, those patients whose blood concentrations were in the lowest decile had 4.4 times the incidence of skin cancer as those in the highest decile (81).

In a 10-year prospective study of 1312 patients with a history of basal cell or squamous cell carcinomas of the skin, Se treatment did not protect against further development of such skin cancers; however, it did reduce total cancer incidence, total cancer deaths, and the incidence of lung, colorectal, prostate, and total non-skin cancer (82,83).

Mechanisms of Action

There is extensive evidence that Se prevents the accumulation of free radicals, thereby protecting from UV damage and fortifying the immune system. Se is an essential cofactor for the intracellular antioxidant enzymes GPX and thioredoxin reductase (TDR) (84). Se is incorporated covalently into proteins of this GPX-TDR family of selenoenzymes (85) as well as into other selenoproteins (86) that may mediate some of the protective effects of Se on UVB-induced cell damage. Through the activities of these enzymes, Se quenches free radicals which would otherwise damage DNA proteins and cellular membranes.

Precise molecular mechanisms are being extensively researched. Protection of keratinocyte DNA was demonstrated by decreased 8-hydroxy-2-deoxyguanosine formation after UV irradiation (87,88), though there was no protection from pyrimidine dimer formation (87). There is evidence that L-selenomethionine (SeMet) induces a DNA repair response in human fibroblasts in vitro (89), perhaps by redox regulation of the DNA repair branch of the p53 pathway (90). In fact, different chemical forms of Se differently modify p53 (each by phosphorylation of specifc cysteine and threonine residues) to induce DNA repair or apoptosis after DNA damage (91). Further cellular protection has been

demonstrated by a decrease in UVB-induced lipid peroxides in keratinocytes (87) and fibroblasts (92) by pre-treatment with SeMet.

Finally, in vitro both SeMet and Se sulfide protect keratinocytes (87,93,94), melanocytes (87,93), and apoptosis (87,95). Interestingly, keratinocytes have twice the GPX activity of fibroblasts which correlates with greatly increased resistance to UVA-induced cell death for keratincytes (96). The fact that Se may prevent UV-induced cell death by p53-independent pathways is evidenced by the demonstration that pre-incubation of cultured human keratinocytes with sodium selenite or SeMet protects from UVB-induced apoptosis without decreasing levels of UVB-induced p53 (97).

Se may also be of particular importance in pigmentation through TDR. Located on keratinocyte membranes, TDR prevents UV oxidation of thioredoxin (which would otherwise enhance tyrosinase synthesis of dihydroxyphenylalanine, the precursor of melanin) (98,99).

Se has other advantageous action on the skin. Clinically, a direct anti-inflammatory effect by oral sodium selenite in Selye granuloma induction in rats was demonstrated (100). This anti-inflammatory action might be a direct result of decreased oxidative damage to cell membranes.

Finally, Se also increases cellular immune responses by several mechanisms, including increasing interleukin IL-2 receptor function (101–103) (thus making cells more resistant to oxidative stress) and through enhanced production of eicosanoids (101).

Effective Topical Formulation

Topical preparations containing Se sulphide are frequently used for the treatment of tinea versicolor, seborrheic dermatitis, and dandruff. However, the Se from these preparations is not absorbed by the skin (104). Se can be effectively absorbed transdermally when applied as SeMet, giving increased skin and liver levels of Se after topical application of 0.02% SeMet to mice (105).

Substantiation of Efficacy

Topical SeMet was shown to be effective in protecting against acute and chronic UV damage to the skin. Concentrations as low as 0.02% increased the MED in humans (106) and decreased acute erythema and blistering as well as later UV-induced tanning and skin cancer in Skh:2 mice (105).

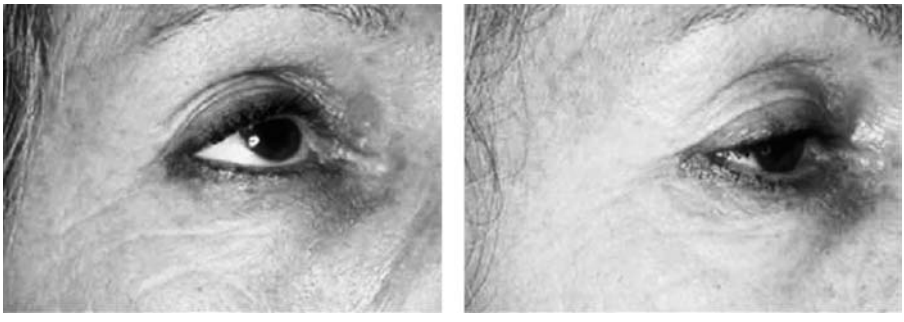


Figure 6 Correction of periorbital wrinkles after four months of once-daily treatment with 0.05% L-selenomethioinine lotion.

Furthermore, topical SeMet is highly effective not only in preventing but also in reversing the appearance of photoaging. As shown clinically in Figure 6, periorbital rhytides are decreased significantly in a 56-year-old woman after four months of once-daily application of SeMet (0.05%) cream.

This enhancement of repair of chronic photoaging at the cellular and molecular level was confirmed by histologic and electron microsopic analysis in mice (107). UV-induced hyperkeratosis and epithelial hyperplasia markedly decreased; irregular, damaged collagen was replaced with newly synthesized, fine fibrillar homogeneous collagen; solar elastosis was repaired; and UV-induced infammation resolved—all as (or more) effectively as comparable treatment with topical tretinoin (107). Electron microscopy confirmed repair of dermal collagen and basement membrane anchoring fibrils.

NEW COMBINATIONS OF ANTIOXIDANTS

Vitamin C with Vitamin E

As shown in Figure 3, the skin uses predominantly vitamin C to protect the aqueous environment and vitamin E to protect membranes from lipid peroxidation. Since vitamin C is naturally present intracellularly in relatively high concentrations, L-ascorbic acid not only acts directly as an antioxidant and as an essential cofactor in the synthesis of collagen, but also regenerates oxidized membrane vitamin E, so that the vitamin E need not be replaced (108). Oral vitamin C with E in high doses protects against UV-induced erythema in humans (109,110) whereas either vitamin alone is less effective (110). Alone each topical L-ascorbic acid (15%) and α-tocopherol (1%) give two-fold protection, whereas combined they provide four-fold protection against UV-induced erythema and thiamine dimer formation in porcine skin (111). This protection from UV-induced erythema (112) and tanning (113) by vitamins C and E combined with melatonin was further demonstrated in humans. Fortunately, mixing these hydrophilic and lipophilic antioxidants in a topical formulation stabilizes each (111) for a cosmetically attractive application.

Vitamins C and E with Ferulic Acid

Ferulic acid is a potent phenolic antioxidant found ubiquitously and in high concentrations in the cell walls of grains, fruits, and vegetables where it is conjugated with mono-, di-, and poly-saccharides and other compounds (114,115). As a potent antioxidant, ferulic acid protects membranes from lipid peroxidation and is synergistic with ascorbic acid (116). Anticarcinogenesis has been demonstrated for pulmonary (117) and colon cancers (118). Topical ferulic was shown to inhibit UVB-induced erythema (119). In a topical preparation, ferulic acid stabilized vitamins C and E and added substantial synergistic photoprotection doubling efficacy as measured by both erythema and sunburn cell formation from four-fold to eight-fold (120). Inhibition of apoptosis correlated with decreased thymine dimer formation and reduced induction of both caspase-3 and downstream caspase-7 (120).

Vitamin E with L-Selenomethionine

In many biologic systems, vitamin E and Se often act synergistically. Borek et al. (121) demonstrated that Se and RRR-α-tocopheryl succinate act alone by different mechanisms to prevent radiogenic and chemically induced transformation in vitro. They further showed that there was additive protection when both were used together (121).

Comparing and combining topical SeMet with oral d-α-tocopheryl acetate and topical d-α-tocopherol (122), the topical combination was less effective than topical vitamin E alone in prolonging the onset and in decreasing the incidence of UV-induced skin cancers in mice (122). Topical SeMet with oral vitamin E was more effective than either alone. In reducing UV-induced pigmentation, topical SeMet with topical or with oral vitamin E was more effective than any one antioxidant alone, particularly during the first eight weeks of UV exposure (122). Topical SeMet (alone or with vitamin E) prevented all blistering after initial UV exposure.

SOY EXTRACT: GENISTEIN

Background

Genistein is an isoflavone isolated from soy, the structure of which is shown in Figure 7. Recent interest in genistein has been stimulated by epidemiological studies which correlate diets high in soy with reduced incidence of cardiovascular disease (123), osteoporosis (123), and certain cancers in humans (124–126).

The direct anticarcinogenic action of genistein is documented. Animal studies demonstrate protection against bladder, breast, colon, liver, lung, prostate, and skin cancer with oral genistein (124,127), and dietary soy inhibits chemically induced skin cancer in mice (128). Growth of many in vitro cancer cell lines is inhibited by genistein (127). Genistein also arrests the growth and induces the differentiation of malignant melanoma cells in vitro (129) and inhibits pulmonary metastases of malignant melanoma cells in vivo (130,131).

Mechanism of Action and Substantiation of Efficacy

The mechanism by which genistein inhibits carcinogenesis may be through inhibition of tyrosine protein kinases, the enzymes which phosphorylate proteins necessary for the regulation of cell division and transformation (132). Of particular importance is phosphorylation of TPK-dependent epidermal growth factor receptors which are related to tumor promotion, including initiation of transcription factors, release of inflammatory mediators (as prostaglandins), and stimulation of cell proliferation (133). Genistein was found to downregulate both UVA- and UVB-induced EGF-R phosphorylation in human epidermoid carcinoma cells in vitro (134,135). In mouse skin, genistein also blocks the UVB-induced expression of the photo-oncogenes c-fos and c-jun which promote cell

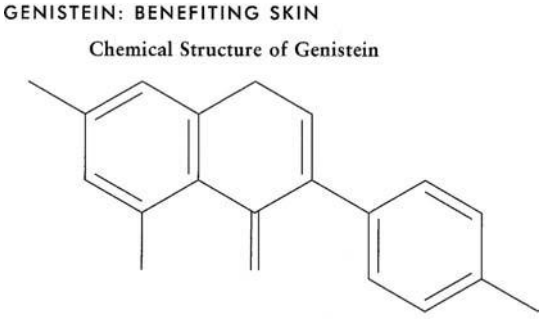


Figure 7 The molecular structure of genistein.

proliferation in oncogenesis (136). Similarly, genistein retards UV-induced apoptotic changes—including caspace-3 and p21-activated kinase 2 activation of human epidermal carcinoma cells (137) and phosphokinase C-delta in human keratinocytes (138).

Genistein is also a potent antioxidant. Genistein scavenges peroxyl free radicals, thereby protecting against lipid peroxidation in vitro (139) and in vivo (140). The decreased incidence of cardiovascular disease with high soy diets may be due to genistein's inhibiting the oxidation of low density lipoprotein (LDL) cholesterol in both aqueous and lipophilic environments. Of direct importance in protection from UV-induced skin damage, genistein has been shown to inhibit in vitro chemical and UV-induced DNA oxidation (141) as well as psoralen plus UVA (PUVA) DNA damage (142,143). The fact that genistein also reduces erythema and histologic inflammation caused by PUVA may have implications for PUVA therapy by reducing possible short- and long-term adverse reactions.

Topical genistein (10 μmol/cm^2) protects against acute and chronic UV damage to the skin (134,135). After exposure of Skh:1 hairless mice to UVB, topical genistein blocked acute skin burns and inhibited UVB-induced cutaneous wrinkling, as demonstrated clinically in Figures 8 and 9 (134,135). Histologic analysis confirmed that topical genistein blocks the signs of chronic photodamage—epidermal hyperplasia and reactive acanthosis with nuclear atypia (Fig. 10) (134,135). At a molecular level, UV-induced damage to DNA (as measured by the biomarker 8-hydroxy-2′-deoxyguanosine) was reduced (144). Inhibition of acute UV-induced erythema with topical genistein (5 μmol/cm^2) was also demonstrated in humans (134,135): Topical genistein (applied 30 minutes before UVB) inhibited by 1 MED the UVB-induced erythema, as shown in Figure 11. Thus, topical genistein may protect human skin against photodamage.

Equally impressive is the fact that topical genistein also inhibits skin cancer, a consequence of chronic UVB damage. Both the incidence and the multiplicity of UVB-induced skin tumors in Skh:2 hairless mice were reduced by about 90% after 25 weeks of UVB exposure (134,135). Figure 12 shows protection from carcinogenesis of representative mice treated with genistein before UVB exposure. Also, after chemical induction and promotion of skin tumors, topical genistein inhibited tumor cell number by 60–75% (144).

Another possible dermatologic benefit of genistein is as a phytoestrogen. The skin has both alpha and beta nuclear estrogen receptors (145) through which estrogen binding can regulate linked genes of proliferation and differentiation. Genistein has a 30-fold

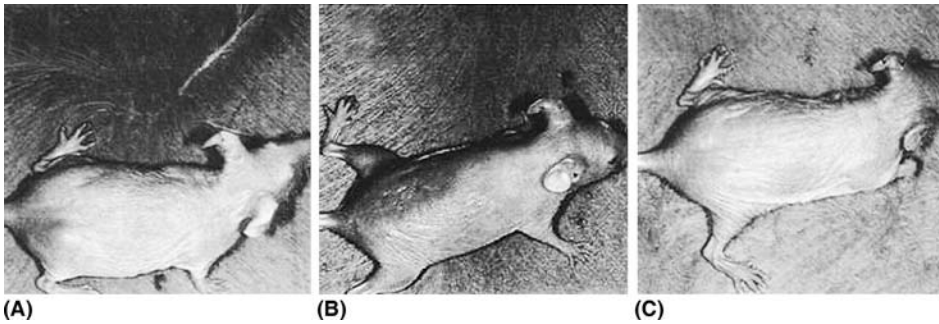


Figure 8 Effect of genistein on UVB-induced acute skin burns in mice were treated topically with 5 μmol genistein 60 minutes before UVB at a dose of 1.8 kJ/cm^2 for 10 days. Photographs were taken 24 hours after last UVB irradiation. (**A**) Negative control (sham irradiation), (**B**) vehicle before UVB, (**C**) 5 μmol genistein before UVB. *Source*: From Ref. 135.

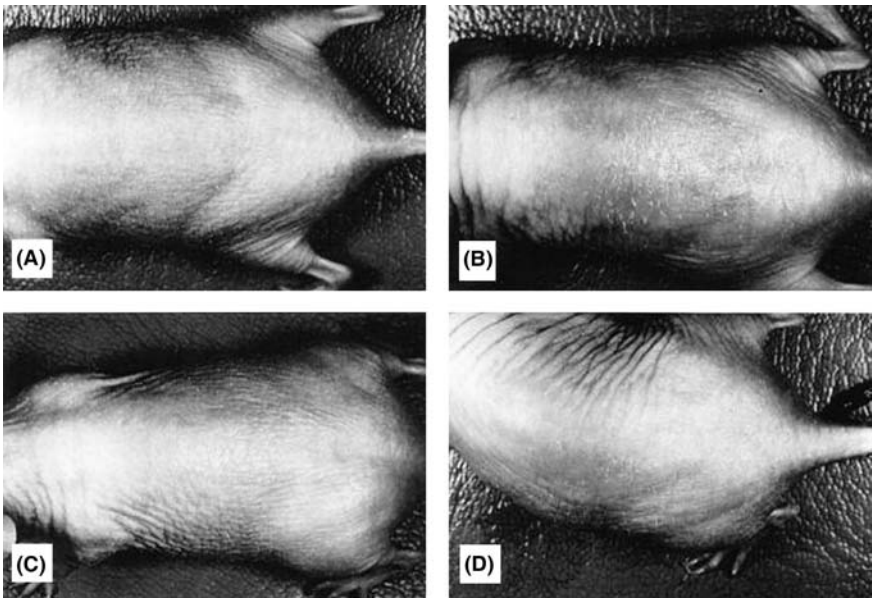


Figure 9 Effect of genistein on UVB-induced chronic photodamage in mice. Skh:1 hairless mice were treated topically with 5 μmol genistein 60 minutes before or five minutes after twice-weekly UVB at a dose of 0.3 kJ/cm^2 for four weeks. Photographs were taken 24 hours after last UVB irradiation. (**A**) Negative control (sham irradiation), (**B**) vehicle plus UVB, (**C**) 5 μmol genistein before UVB, (**D**) 5 μmol genistein after UVB. *Source*: From Ref. 135.

higher affinity for ER-beta than ER-alpha (146), but a greater ER-alpha agonist activity than ER-beta (147). Though estradiol has 700-fold more ER-alpha and 45-fold more ER-beta activity than genistein, the possible biologic effect of genistein through dietary soy isoflavones may be important. Oral (148,149) and topical estrogen (150,151) increase the collagen content of skin which diminishes with aging. This effect is especially dramatic in women during and after menopause (152). Genistein may reduce the atrophic appearance

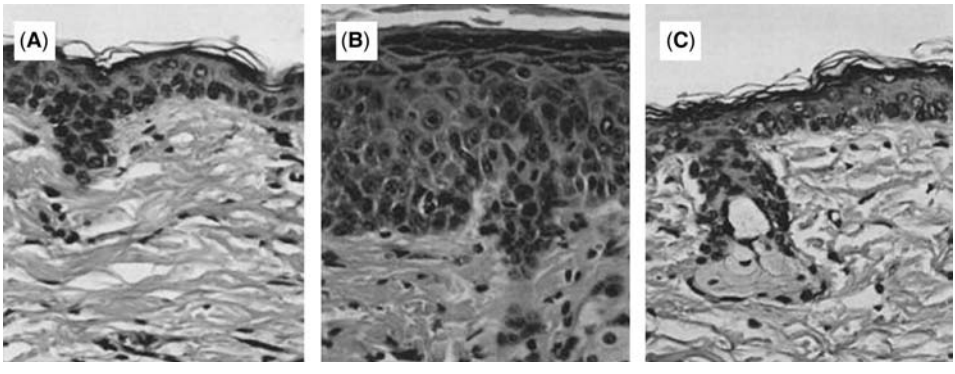


Figure 10 Effect of genistein on histological alterations in mice exposed to UVB. Skh:1 hairless mice were treated topically with 5 μmol genistein 60 minutes before UVB at a dose of 0.3 kJ/cm^2 twice weekly for four weeks. Mice were killed 24 hours after the last UVB irradiation and skin specimens were taken for histology. (**A**) Negative control (sham irradiation), (**B**) vehicle plus UVB, (**C**) 5 μmol genistein before UVB. *Source*: From Ref. 135.

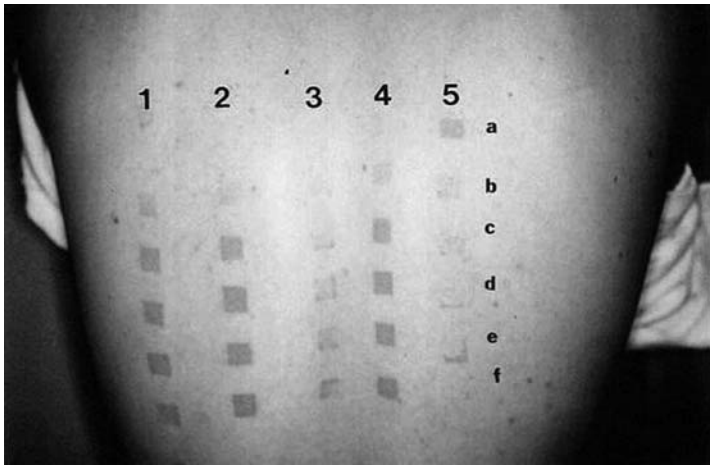


Figure 11 Effect of genistein on UVB-induced erythema in human skin. The study was performed in the phototherapy unit in the Department of Dermatology, Mount Sinai Hospital. UVB fluences used a range from 0 to 100 mJ/cm^2. Genistein was applied to dorsal skin either 60 minutes before or five minutes after UVB exposure. Photographs were taken 24 hours after UVB irradiation. A minimal erythema dose (MED) for this individual was 40 mJ/cm^2. Lane 1: Vehicle before UVB; lane 2: no treatment before or after UVB; lane 3: 1μmol genistein/cm^2 of skin before UVB; lane 4: 1μmol genistein/cm^2 of skin after UVB; and lane 5: dose response of topical genistein applied before UVB (1 MED) at a dose ranging from 0.05 to 5 μmol/cm^2. *Source*: From Ref. 135.

of aging skin both by preventing photodamage through inhibition of metalloproteinases in human skin (independent of sunscreen effect) and by stimulating collagen synthesis. Indeed, genistein does increase collagen gene expression in fibroblasts in vitro (153).

Thus, topical genistein shows promise not only in protecting the skin against acute and chronic photodamage but also in enhancing the diminished collagen synthesis of normal intrinsic aging.

Challenges in Formulation

As described above, topical 5 μmol genistein has been studied extensively and has been proven to protect from UV damage. Unlike vitamin C, genistein is a stable molecule. Unlike vitamin E and Se, genistein is absorbed transcutaneously to give protective activity. The only challenge in formulation is to have a pure source of genistein without other soy contaminants.

ALPHA-LIPOIC ACID

Background

R-Alpha lipoic acid (α-LA) is synthesized in the mitochondria of plants and animals, including humans. Natural α-LA is covalently bound to proteins via lysine; thus only minimal free α-LA enters the circulation after biosynthesis or eating α-LA-rich food (22). The lipoamide is a required co-factor for two enzymes in the citric acid cycle. It is also essential for the formation of a cofactor required in nucleic acid synthesis and for the metabolism of branched-chain amino acids.

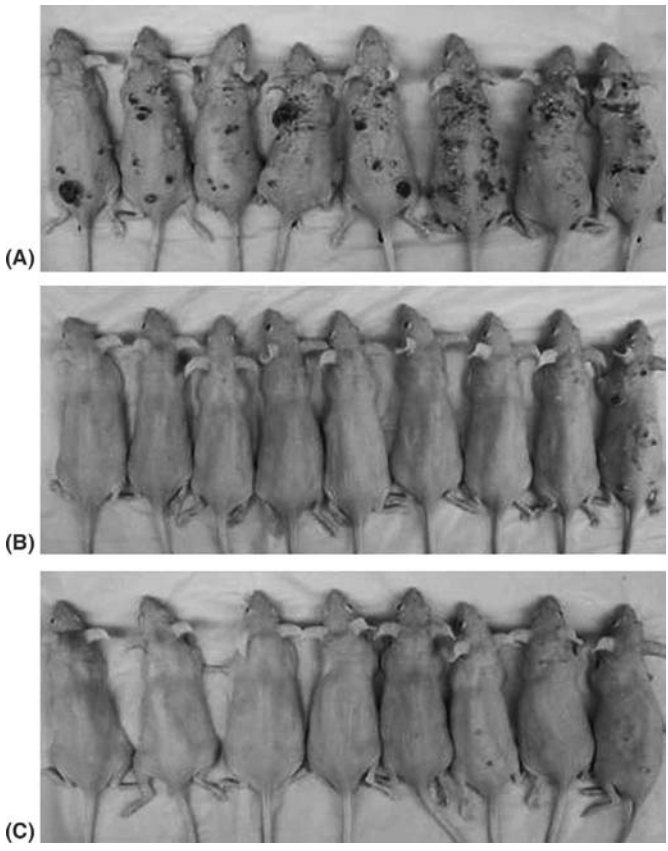


Figure 12 Representative photograph of inhibition of photocarcinogenesis in mice treated with genistein. (**A**) Hairless mice irradiated with 0.3 kJ/m^2 thrice weekly for 25 weeks. (**B**) Mice treated with 1 μmol genistein before UVB exposure. (**C**) Mice treated with 5 μmol genistein before UVB irradiation. *Source*: From Ref. 135.

With oral supplements of free α-LA, unbound α-LA is transported to tissues (22). Free α-LA is rapidly metabolized by the liver, so that the half-life in blood after absorption is only about 30 minutes, limiting the amount delivered (22). High tissue levels are short-lived since most free α-LA is rapidly reduced to dihydrolipoic acid (DHLA), as shown in Figure 13 (21,22).

Notwithstanding this transient availability, free α-LA has been shown to be therapeutic for autoimmune liver disease by binding autoantibodies, heavy metal intoxication by trapping circulating metals, diabetic polyneuropathy by preventing oxidative damage, and mushroom poisoning (22). Although not normally found in significant amounts in the skin, α-LA is a good candidate for topical application (21,154):

- As a small, stable molecule, it could successfully be percutaneously absorbed.
- As a potent antioxidant it might protect from UV and other free radical environmental changes;
- Because it is soluble in both aqueous and lipid environments, it can interact with oxidants and antioxidants in many cellular compartments.

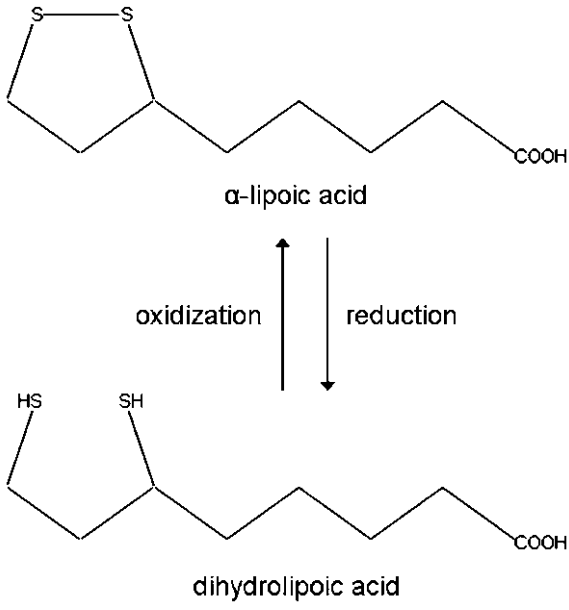


Figure 13 The molecular structures of α-lipoic acid and dihydrolipoic acid.

Mechanisms of Action

Topical α-LA with its metabolite DHLA could protect the skin from oxidative stress in several ways. Both α-LA and DHLA are highly effective antioxidants, as summarized in Table 1 (22). DHLA is actually the more potent form. Both successfully scavenge ROS in vitro and in vivo. However, pro-oxidant activity has been observed. This occurs when an antioxidant reacts with a ROS scavenger, forming a product that is more harmful than the scavenged ROS. Fortunately α-LA can act as an antioxidant against the pro-oxidant activity of DHLA (22). Both α-LA and DHLA further provide antioxidant activity by chelating Fe2+ and Cu2+(α-LA) and Cd2+ (DHLA) (22).

DHLA, unlike α-LA, has the capacity to regenerate the endogenous antioxidants vitamin E, vitamin C, gluthatione, and ubiquinol, as illustrated in Figure 3. This is clearly

Table 1 Antioxidant Activity of α-Lipoic Acid and Dihydrolipoic Acid (DHLA)

	α-Lipoic acid	DHLA
Antioxidant	+	++
Scavengers reactive oxygen species (ROS)	+	+
Chelates metals: Fe^{2+}, Cu^{2+}	+	−
Cd^{2+}	−	+
Regenerates endogenous antioxidants (vitamin E, vitamin C, glutathione, ubiquinol)	−	+
Repairs oxidatively damaged proteins	−	+
Pro-oxidant	+	+

Abbreviations: +, indicates activity; ++, indicates greater activity; −, indicates no activity.
Source: Adapted from Ref. 22.

of great importance for skin, since UV exposure directly depletes especially ubiquinone and vitamin E as well as vitamin C, thereby stressing the other linked antioxidants (154). Regeneration of these major membrane and cytosol antioxidants gives cascading protection. Increases in the other important antioxidants (intracellular glutathione and extracellular cysteine) are noted when α-LA is added to cell cultures (22). Vitamin E deficient animals do not show symptoms (weight loss, neuromuscular abnormalities) when supplemented with α-LA (155).

Although α-LA is a potent antioxidant, it provides no effective protection against UV-induced erythema or cell damage measured as sunburn cells (156). However, α-LA (but not DHLA) acts as an anti-inflammatory agent by reducing the production and inhibiting the binding of transcription factors such as nuclear factor-kappa B (NF-kappa B), thereby indirectly affecting the gene expression of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukins (157). DHLA (but not α-LA) can repair oxidatively damaged proteins, which in turn regulate the activity of proteinase inhibitors such as α l-AP, an inflammatory modulator (158). As antioxidants, both α-LA and DHLA are directly anti-inflammatory by virtue of their quenching oxidants secreted by leukocytes and macrophages at sites of inflammation (158).

α-LA may prove to retard and correct both intrinsic and extrinsic aging of the skin as well as other organs (159). By damaging DNA, the ROS continuously formed in normal metabolism may be largely responsible for the functional deterioration of organs with aging. A decrease in cellular protein and DNA as well as in α-LA levels has been measured in aged rat liver, kidney, and spleen (160). Supplementation with α-LA increases nucleic acid and protein levels in the elderly organs (160). Similarly, the age-related decrease of mitochondiral function in cardiac and brain cells can be improved with α-LA supplementation (161). Clearly, aging skin might similarly benefit.

Formulation

α-LA has been found to penetrate rapidly into murine and human skin to dermal and subcutaneous layers. Two hours after application of 5% α-LA in propylene glycol, maximum levels of α-LA were attained in the epidermis, dermis, and subcutaneous tissue (154). The stratum corneum concentration of α-LA predicted the penetration and levels in the underlying skin. 5% of the α-LA was converted to DHLA in both the epidermis and dermis, leading the researchers to conclude that both keratinocytes and fibroblasts reduce α-LA (154).

Efficacy

To evaluate possible improvement to photodamage, a split-face study was done on 33 women (162). Topical application twice daily of 5% lipoic acid cream for 12 weeks decreased skin roughness by 50.8% (as measured by laser profilometry) when compared with the placebo. Clinical and photographic evaluation showed reduction in lentigenes and fine wrinkles in this and one other study (163). Clearly, topical α-LA should be further investigated by quantitative techniques to confirm these results and to elucidate mechanisms of action.

UBIQUINONE

Background

Ubiquinone (coenzyme Q10, Figure 14) is so named because it is ubiquitous in virtually all living cells, excluding some bacteria and fungi, although the level is quite variable. Since most human tissues synthesize ubiquinone, it is not considered to be a vitamin.

Ubiquinone is primarily located in the inner mitochondrial membrane where it is essential for the production of the ATP required for all vital cellular functions (164). Until recently, ubiquinone was thought to function only in energy transduction; however, with the discovery that ubiquinone is also an antioxidant within subcellular membranes, new roles are now being recognized. Ubiquinone can regenerate reduced tocopherol, as depicted in Figure 3. In fact, within membranes the amount of ubiquinone is from three to thirty times that of tocopherol (165). Without ubiquinone, the regeneration of tocopherol would be very slow (166,167).

Mechanisms of Action

The fact that ubiquinone can serve not only as an energy generator but also as an antioxidant in the skin has been investigated (168,169). In cultured human keratinocytes exposed to hydrogen peroxide, the detrimental increase in the activity of phosphotyrosine kinase was suppressed and the loss of glulathione was prevented (169). Ubiquinone (0.3%) also suppressed the UVA-induced reduction of mitochondrial membrane potential in fibroblasts from both young and old human donors (169). Finally, the UV-induced oxidative damage to DNA in keratinocytes in vitro was reduced significantly with ubiquinone (169).

Ubiquinone can retard loss of hyaluronic acid and slowdown of cell division—both manifestations of intrinsic aging. Aged human fibroblasts in vitro produce less glycosaminoglycan and proliferate more slowly than young cells. The addition of ubiquinone increased levels of glycosaminoglycan as well as rates of cell division (169).

Ubiquinone further protects from the UVA-induced degradation of collagen. Both ubiquinone and vitamin E were shown in vitro to suppress fibroblast production of UVA-induced collagenase, thereby markedly retarding collagen breakdown (169). Ubiquinone suppressed collagenase expression over a longer period of time than did vitamin E.

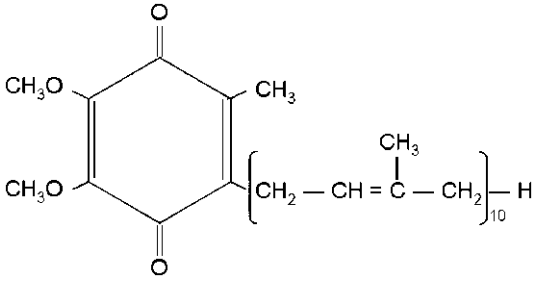


Figure 14 The molecular structure of ubiquinone. The "head" of the ubiquinone molecule is a fully substituted quinone ring which does not allow addition reactions with thiol groups in the cell (such as GSH). Ubiquinones vary by the length of the "tail": Q10 has 10 isoprene units. Humans can synthesize Q10 out of the other coenzymes Q1 to Q9, though this ability decreases with age.

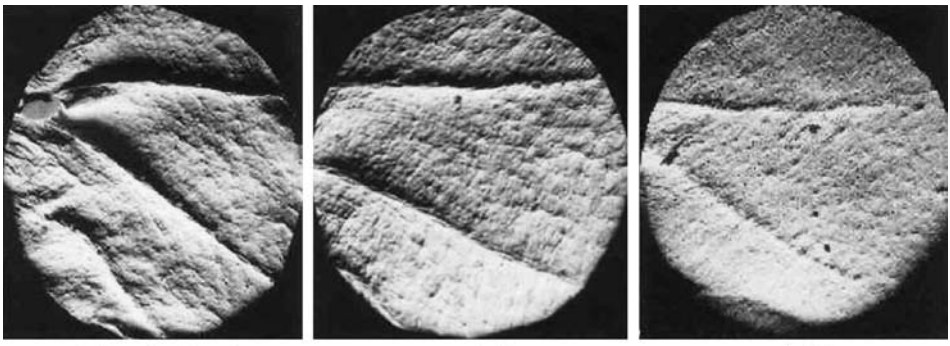


Figure 15 Reduction of wrinkles with ubiquinone. Silicone replicas of the skin, analyzed by laser profilometry, show a significant reduction in the depth of periorbital fine lines and wrinkles in a 46-year-old female after 10 weeks of twice-daily application of ubiquinone cream (Eucerin Q10 Anti-Wrinkle Sensitive Skin Creme). *Source*: From Ref. 168.

Formulation

The concentration of ubiquinone is highest in organs with high rates of metabolism such as heart, kidney, and liver, where it functions as an energy transfer molecule (164). In skin, the level of ubiquinone is relatively low, with 10-fold higher levels in the epidermis than in the dermis (169). Thus, the epidermis might potentially benefit from topical ubiquinone. Indeed it has been demonstrated that ubiquinone can be topically absorbed. Application of ubiquinone in ethanol to porcine skin achieved 20% penetration into the epidermis and 27% into the dermis (169).

Substantiation of Efficacy

Ubiquinone's antioxidant action in skin was confirmed in vitro by sophisticated ultra-weak photon emission (UPE) (169). Increased antioxidants result in decreased UPE. Elderly volar skin demonstrated 33% reduction in antioxidant activity when compared with young skin. This was corrected after one week of twice-daily topical application of 0.3% ubiquinone. After UVA irradiation, a decrease in antioxidant activity was noted; this loss was significantly corrected with topical 0.3% ubiquinone.

The efficacy of ubiquinol in reversing photoaging was further studied clinically (168). Ubiquinol cream (0.3%) was applied to one-half of the face and placebo to the other once daily for six months. Casts were made of the periorbital rhytides. The improvement can be appreciated in the photographs shown in Figure 15. Quantitative microtopography demonstrated a 27% reduction in the mean wrinkle depth.

Another clinical measure of photoaging is stratum corneum cell size. With deceased cell turnover time in aged skin, comeocytes become larger. Treatment once daily for six months with ubiquinone cream decreased corneocyte size equivalent to rejuvenation of 20 years (168). Thus, ubiquinone is be an effective antioxidant protecting the dermal matrix from both intrinsic and extrinsic aging, making it a potentially important cosmeceutical.

SUMMARY

Nutritional antioxidants represent a novel category of cosmeceuticals. There is no doubt that higher levels are achieved in the skin through topical application than with oral

supplementation, thus providing a protective antioxidant reservoir in the skin. Current research indicates that topical vitamin E and C and L-SeMet provide UV photoprotection and reverse photoaging. Ubiquinone and genistein may provide photoprotection. In addition, they as well as topical α-lipoic acid may retard both intrinsic aging and photoaging. There is further evidence that α-lipoic acid and ubiquinone may also reverse photoaging. Thus, topical antioxidants continue to be an important area of cosmeceutical research.

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What Is Next in Skin Care Cosmetic Products?

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The cosmetic industry has changed dramatically over the past 20 years with the introduction of daily UV lotion in the late 1980s to fight future aging. No longer are women searching for hope in a jar but focusing on the latest over-the-counter breakthrough products with clinically demonstrated biological activity. This quest for skin health and youthful beauty has driven many consumers to explore a variety of approaches. It has also triggered a renaissance in the world of skin care where health, beauty, and technology are converging to create new and exciting opportunities.

This frantic search for beauty and youth has stimulated a remarkable growth in the skin care industry. Skin care advances are moving quickly as they mirror advancing technology in pharmaceuticals and biotechnology. Global retail sales of anti-aging skin care products have increased 71% since 2000 (1). In 2004 skin care sales topped $12 billion, with $7 billion of that being spent on facial treatments alone (2). As biotechnological and pharmaceutical research continues to result in technologic advances, skin care companies will continue to spend millions of dollars on incorporating these advances into skin care products. The average woman will find more choices to aid her in the battle against aging, including customized products and new novel ingredients with increased effectiveness and more precise delivery.

COSMECEUTICALS

Clearly cosmeceuticals are the fastest growing segment of the skin care market (3) and are currently the driving force in the field of skin care research (4). Cosmeceuticals are cosmetics that contain biologically active ingredients, and while these ingredients are not classified as drugs, they do have documented functional treatment benefits. When cosmeceuticals are labeled and marketed as cosmetics, they are not regulated by the FDA.

Cosmeceuticals are used primarily to combat the effects of aging on the skin. More women are yearning for healthy, youthful skin, fueling the demand for these anti-aging products. Younger women are also looking to these products as a preventive strategy against aging. Cosmetic companies are investing millions of dollars to develop new and

better actives for anti-aging products, and women of all ages are constantly trying the newest product and consulting their dermatologists for therapeutic approaches to fight the signs of aging.

Retinoids are the most recognized anti-aging ingredient, comprising a family of compounds with structures and mechanisms of action that resemble those of vitamin A. Retinoids are essential nutrients which play a role in cell growth and differentiation (5). Tretinoin, the most popular retinoid, increases dermal collagen, cellular differentiation, and proliferation. It has been shown to improve skin's global appearance, particularly affecting fine and coarse wrinkling, roughness, pigmentation, and sallowness (6,7). However, tretinoin is a drug regulated by the FDA. Retinol, first generation retinoid, is often added to over-the-counter cosmetics (8). Retinol must be converted to retinaldehyde and then to all-trans-retinoic acid within the keratinocyte to become active (9). Because retinol is a cosmetic ingredient, it is not labeled as an active ingredient. While not labeled as such, many published studies demonstrate the significant biological action and efficacy of this cosmetic vitamin A derivative. Retinoids and other alternate metabolisms of vitamin A will continue to be key mainstay cosmeceutical ingredients.

Another popular cosmeceutical affecting cellular proliferation is alpha-hydroxy acid (AHA). AHAs increase the type I collagen, mRNA, and hyaluronic acid content of the epidermis and dermis (3). They also renew the stratum corneum by promoting desquamation. Glycolic acid, lactic acid, and malic acid are all examples of AHAs. Newer generation polyhydroxy acids are also being studied; these PHAs provide additional moisturization compared to AHAs, and do not cause the irritating response associated with AHAs (10). They also possess antioxidant properties (10).

A major class of cosmeceutical ingredients is antioxidants that mediate free-radical damage from UV radiation. Since the skin's own supply of free-radical scavengers is limited, topical antioxidants, which scavenge free radicals and protect cells from damage, can attenuate skin damage from UV radiation. Topical antioxidants include vitamins C and E, alpha-lipoic acid (ALA), and coenzyme Q10. In addition to their antioxidant effects, these agents all have other documented anti-aging properties. Vitamin C has collagen stimulating properties and has been shown to be photoprotective (4). Vitamin E decreases free-radical production as well as inhibits collagenase production (11). ALA is a strong intracellular free-radical scavenger (12). It also has anti-inflammatory action, inhibiting the production of pro-inflammatory mediators (3). Coenzyme Q10 (ubiquinone) is present in every cell in the body and acts as a coenzyme in energy production. It has also been shown to improve skin texture (13). One of the bigger challenges to the future use of antioxidants is assuring biological activity from a cosmetic preparation and measuring the antioxidant benefit in a clinical environment. As these challenges become resolved, a significant increase in use and benefit of these ingredients is expected.

The renewed focus on health in today's society has also created a niche for natural and organic products. Women are interested in natural ingredients that make therapeutic claims. This has led to increased popularity of skin care products containing plant or mineral ingredients, especially in the spa market. Organic advocates are willing to pay extra for skin care products that are clearly organically produced (14). Therefore, one of the hottest areas for cosmeceutical ingredients is the utilization and understanding of botanicals. Topical botanicals have been shown to combat reactive oxygen species, as well as often having various secondary effects. Some strong botanicals include tetrahydrocurcumin, pycnogenol, silymarin, and soy extracts (15). The usage of botanicals for their anti-inflammatory function continues to grow. Botanicals have been shown to block inflammatory changes that may result in cutaneous aging. Some common anti-inflammatory botanicals include aloe vera, green tea, and allantoin (15). However, some

newer research suggests the molecular structure, as well as the formulation delivery system, strongly affects the biological activity of botanicals. Understanding the effect and potential of botanicals as cosmeceutical ingredients will likely continue to be a key industry focus.

There are several different types of growth factors of both plant and animal origin that have been incorporated into cosmeceuticals. Furfuryladenine (kinetin), a synthetic plant growth factor that delays senescence of plant cells, has shown in vitro benefits in retarding cellular aging (16). Transforming growth factor-beta 1 is an important human growth factor with therapeutic potential because of its role in neocollagenesis (3). Human growth factors are relatively under explored by the cosmetic industry today and given the negative public view associated with this class of ingredients it is unlikely that they will be a top focus area in the coming years.

Stimulating the skin's natural repair and rejuvenation system by topically adding skin functional ingredients like peptides, hyaluronic acid, niacinamide (vitamin B3), estrogen, and dimethylaminethanol will continue to show promise in improving the appearance and texture of skin. Delivering these relatively large molecules to the biological key targeted area to maximize the effect remains the key barrier to skin aging damage reversal or stimulation. Research in this area will continue with the next wave of cosmeceutical ingredient breakthroughs.

NUTRACEUTICALS

Nutraceuticals provide beauty benefits from the inside out; their goal is to enhance beauty by improving health. There are several dietary supplements that have been developed to promote skin health in particular. These supplements provide vitamins and nutrients especially involved in skin physiology. The challenge for the nutraceutical industry is to definitively measure the benefit of these oral supplements in clinical testing. In the future it is expected that more published clinical data will be available, as well as industry-regulated labeling systems to describe the claimed benefits.

MEDICAL MIMICS

The growing demand for anti-aging products has led to the development of "medical mimics." These are new cosmetic alternatives to costly dermatologic procedures and surgeries. "Facial relaxers" are gaining popularity as an alternative to Botox injection. Argireline, a synthetic peptide that has been touted to relax facial muscles by inhibiting the neurotransmitter catecholamine, has been advertised as having impressive wrinkle reduction effect (17). Several companies have developed home products that mimic microdermabrasion. These products use lower dose crystals and sometimes a warming agent to smooth, polish, and resurface the skin, producing results similar to office dermabrasion. Utilizing lower levels and less aggressive chemical peel acid ingredients, at-home chemical peels have also become popular. While home laser treatments are not yet available, it is expected that low dose home lasers are in the not so distant future for skin texture improvement and hair removal. It is expected that the "medical mimic" trend will continue as women try to balance their busy lives.

CUSTOMIZED PRODUCTS

The genomics revolution has already begun to transform the pharmaceutical industry, and it is now making its mark on the cosmetic industry as well. At the heart of this revolution is the ability to generate and assemble massive amounts of DNA sequence information. We are now able to identify key genes in biological processes such as skin aging through a method called gene expression profiling. Single nucleotide polymorphisms (SNPs) represent the genetic basis for inter-individual differences in disease susceptibility, including aging. The identification and mapping of these SNPs is an area of active biotechnologic research.

As a result of these advances, two promising applications of the genomics revolution are beginning to develop: (i) the use of an individual's DNA sequence information as the basis for the development of improved clinical study design and preventative and diagnostic strategies and (ii) the use of DNA sequence information to develop personalized medicines and products. There are several factors that will influence when and how the DNA sequencing will be applied to the development of cosmetic products (18). These include the progression of the science, consumers' willingness to use their DNA sequence for product choices, and market considerations. Ideally, this genetic technology will allow cosmetic companies to identify specific skin qualities—such as texture, pigmentation, hydration, and wrinkles—and alter products to meet individual skin needs.

SKIN TONE ALTERATION

Skin tone is an area of dissatisfaction for many women around the world. Clear, fair skin tones are the goal in Asia, and skin lighteners have been popular there for many years. However, they are now gaining popularity in the west as well. They can also be used to treat disorders of hyperpigmentation, such as age spots. Tyrosinase is a key enzyme in the production of melanin. Phenolic skin lightening agents such as hydroquinone interfere with melanogenesis by acting as competitive inhibitors of tyrosinase, so that the skin is less pigmented. Non-phenolic skin lightening agents, including glucosamine, kojic acid, azelaic acid, and licorice extract, also inhibit tyrosinase activity. Skin lightening agents are now being incorporated into bar soaps and color cosmetics as well.

In western countries, where darker skin is often idealized, self-tanners continue to increase in popularity. These usually contain dihydroxyacetone, which reacts with keratin protein in the stratum corneum to form melanoidins to give the temporary brown color to the skin. Because the stratum corneum is continually sloughed, the results are temporary. Manufacturers continue to work toward developing self-tanners that are odorless, quick to dry, and unlikely to streak (19). They also are working to improve delivery systems, including wipes, sprays, and foams.

Optical technology is now being incorporated into products to improve the appearance of skin. These new products do not change the skin at all, but when they are applied to the skin, they improve its appearance. The basis for this technology is that tiny particles can reflect and emit visible light from the skin. When used in cosmetics, the resultant reflected light can help hide wrinkles, large pores, and even cellulite and make the skin appear healthier (20).

Cosmetic companies continue to actively research and promote products to decrease cellulite. Ingredients such as caffeine, kiwi and green apple extracts, shiitake mushroom extract, gingko biloba, and seaweed extracts are all being incorporated into products

intended to firm the skin, increase elasticity, and decrease cellulite (21). Although none of these products have delivered the cure, women everywhere continue to have hope.

DELIVERY SYSTEMS

Active research continues in the area of delivery systems for cosmetic products. Particulate delivery systems such as liposomes, which are tiny, hollow lipid spheres, are used to carry active ingredients into the skin. However, smaller, more specialized transportation systems are being developed; these include nanoparticles, microcapsules, and millicapsules.

Nanotechnology is making its way to the forefront of the cosmetic industry. Nanoparticles are solid hydrophobic spheres with an average particle size of less than one micron; they have high cationic charge density to improve their deposition onto the target site and prevent them from being washed off during rinsing (22). This bioadhesive quality also reduces the need for reapplication. The hydrophobic quality of the nanospheres sustains the diffusion rate of the active ingredients, which allows their release over an extended period of time. The nanospheres have improved stability when compared with emulsion-based delivery systems, such as liposomes. This enhanced stability prolongs product shelf life. In addition, the substance to be delivered does not have to be soluble in the vehicle, since it can be dispersed in the solid matrix. Incorporating an ingredient such as a sunscreen into nanoparticles in a skin care product allows the product to block UV light, but does not interfere with the look and feel of the lotion. As nanotechnology advances, it may enable the development of more customized and effective personal care products.

NEW USERS

Male grooming is one of the fastest growing sectors in the cosmetic industry (23). There are significant differences between men's and women's skin; men's skin tends to be less acidic, thicker, oilier, and hairier (24,25). By using products developed specifically for their skin type, men will achieve better results. Products being developed particularly for men include not only moisturizers, but also products to combat aging, self-tanners, blemish-control products, concealer products, and bath and shower products. These cosmetic products will be developed and promoted to seem masculine, so that the average male will feel comfortable using them. Consistent with this trend it is expected there will be an increase in male visitors to the dermatologist's office for cosmetic procedures.

THE SKIN CARE MARKET

More effective anti-aging ingredients and formulations are being developed every day. Cosmetic alternatives to dermatological procedures will be increasingly available for the average woman, and technical innovations to cosmeceuticals will allow skin care products to deliver active ingredients more effectively and with greater precision. Emerging genetic-based technology will enable the development of targeted products that are customized to meet the needs of today's individual man or woman. In addition, the growing concern for personal health will further expand the nutraceutical market. With the increase in consumer expectations and the continuation of changing trends, the collaboration between the dermatology professional community and skin care product innovators must continue to be fostered.

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