
Topical L-Ascorbic Acid: Percutaneous Absorption Studies

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BACKGROUND. Reactive oxygen species generated by ultraviolet light result in photocarcinogenic and photoaging changes in the skin. Antioxidants protect skin from these insults.

OBJECTIVE. This study defines formulation characteristics for delivering L-ascorbic acid into the skin to supplement the skin's natural antioxidant reservoir.

METHODS. L-ascorbic acid or its derivatives were applied to pig skin. Skin levels of L-ascorbic acid were measured to determine percutaneous delivery.

RESULTS. L-ascorbic acid must be formulated at pH levels less than 3.5 to enter the skin. Maximal concentration for optimal percutaneous absorption was 20%. Tissue levels were saturated after three daily applications; the half-life of tissue disappearance was about 4 days. Derivatives of ascorbic acid including magnesium ascorbyl phosphate, ascorbyl-6-palmitate, and dehydroascorbic acid did not increase skin levels of L-ascorbic acid.

CONCLUSIONS. Delivery of topical L-ascorbic acid into the skin is critically dependent on formulation characteristics.

IN THE PRESENCE of our oxygen-rich atmosphere, ultraviolet light generates reactive oxygen species in skin. In addition to sunlight, other inflammatory insults including smoking and pollution generate reactive oxygen species. Reactive oxygen species, in turn, cause oxidation of nucleic acids, proteins, and lipids. Reactive oxygen species alter DNA,¹⁻⁷ as well as its repair,⁸ and trigger cytokine cascades that result in photoaging^{9,10} and photocarcinogenesis.¹¹

The body protects itself naturally from reactive oxygen species by using antioxidants to neutralize them before they cause damage to the skin and its components. Vitamin C, or L-ascorbic acid, is the most abundant antioxidant in skin.¹² Although most plants and animals synthesize L-ascorbic acid to protect themselves from free radical attack, a gene necessary for its synthesis, L-gulonolactone oxidase, has been mutated in humans.¹³ As a result, humans rely on dietary intake for their supply.¹⁴ L-ascorbic acid is quite water soluble and serves as the major aqueous phase reductant in the body.¹⁵

Since skin relies on antioxidants for protection against reactive oxygen species, and since skin predominantly receives and must deal with the free radical assault resulting from UV light, increasing the antioxidant defense of skin becomes an attractive strategy for increased photoprotection.¹⁶ If antioxidants could be delivered in high concentration through the stratum corneum barrier into the skin, then the antioxidant protective reservoir could be increased and photoprotection might be enhanced. Indeed, our laboratory has described a stable aqueous formulation of L-ascorbic acid that gets into skin and provides photoprotection against both UVB and UVA-psoralen phototoxicity by a mechanism that is clearly not a sunscreen effect.¹⁷ Moreover, we have demonstrated that topical L-ascorbic acid protected against UV immunosuppression and tolerance to contact antigen in mice.¹⁸

In order to maximize the protective effects of topical L-ascorbic acid in skin, we have undertaken this study of formulation composition and kinetics so that we can maximize the amount of L-ascorbic acid delivered into the skin.

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Materials and Methods

L-ascorbic acid (pharmaceutical grade) was purchased from Roche (Nutley, NJ). All concentrations of L-ascorbic acid were made fresh and stabilized in 2% ZnSO₄, 0.5% bioflavonoids, 1% hyaluronic acid, 0.1% citrate in glass-distilled water. pH was adjusted with triethanolamine. Commercial formulations of 13% magnesium ascorbyl phosphate (Vivi-

fying Serum C, Dr. Mary Lupo Skin Care Products, New Orleans, LA) and 10% ascorbyl-6-palmitate (C-Esta Serum, Jan Marini Skin Research, San Jose, CA) were obtained fresh from the manufacturer and opened just prior to testing. Samples were tested as is; the content was not confirmed. Dehydroascorbic acid 1 M (17.4%) was prepared by taking 1 ml of a 1 M ascorbate solution, adding bromine 55 μ l, vortexing vigorously for 30 seconds, and then immediately bubbling with nitrogen for 10 minutes.¹⁹ The institutional review board of the College of Veterinary Medicine at North Carolina State University approved the animal experiments.

Experiments were conducted in white Yorkshire pigs. The skin was shaved with an electric shaver 24 hours before the experiment began to allow healing of any skin nicks. Two hundred μ l of formulation, the maximal volume of the chamber, was placed under a Hill Top Chamber (Hill Top Co., Cincinnati, OH) for 22–24 hours. The chamber is semi-occlusive and protects the material from smearing. Although the chamber may enhance percutaneous absorption, in several cases in the experimental results, the baseline skin levels of L-ascorbic acid were not increased. In some experiments the chamber was replaced with fresh solution and changed each day. In clinical practice, vitamin C solutions are usually applied daily. At the end of the experiment, the formulation was washed vigorously from the skin with water. Washed skin was tape stripped 15 times to remove surface contamination and stratum corneum. The tape strips were discarded. Studies have shown that tape stripping removes the stratum corneum layers (data not shown) and removes surface radioactivity of topically applied substances bound to the stratum corneum.^{20,21} Full-thickness 6 mm punch biopsy specimens of skin were taken and placed immediately into liquid nitrogen. Tissue was shattered in liquid nitrogen and weighed aliquots extracted and stabilized in 60% methanol, 1 mM EDTA in water, centrifuged, and kept at -70°C until analyzed. Samples were analyzed in a blinded manner for vitamin C by high-performance liquid chromatography (HPLC) with coulometric electrochemical detection.^{22,23} The method is specific for L-ascorbic acid and has a sensitivity of 50 fmol. The results are expressed as mean \pm standard deviation. Unless otherwise noted, $n = 3$. The P values were calculated by two-tailed Student's t -test with equal variance.

Results

pH

Fifteen percent formulations of L-ascorbic acid were tested at pH levels between 2.0 and 5.0 (Figure 1). Tissue levels of L-ascorbic acid were enhanced only at formulation pH levels less than 3.5. The pKa for L-ascorbic acid is 4.2. Apparently the molecule must be un-ionized for percutaneous absorption to occur. Low pH is essential for absorption and delivery is enhanced as the pH is reduced to 2.0. The effect of the solutions on skin pH is unknown.

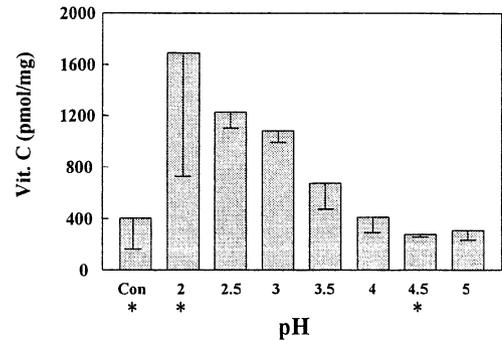


Figure 1. Effect of pH on percutaneous absorption. 15% L-ascorbic acid at different pHs were applied to pig skin for 24 hours. Skin levels of L-ascorbic acid are expressed as mean \pm SD ($n = 3$). *Average ($n = 2$).

Concentration

L-ascorbic acid concentrations were tested from 5 to 30.0% (Figure 2). pH was adjusted to 3.2. Tissue levels of L-ascorbic acid increased and were maximal at 20%. For unknown reasons, concentration levels higher than 20% resulted in decreased tissue levels.

Kinetics

Fifteen percent L-ascorbic acid at pH 3.2 was applied daily for 1–5 days (Figure 3). After 3 days, tissue levels were apparently saturated. Levels achieved were approximately 20 times normal tissue levels.

Washout

Fifteen percent L-ascorbic acid at pH 3.2 was applied daily for 5 days to saturate skin levels (Figure 4). Skin levels of L-ascorbic acid were then measured at daily intervals, with no further topical application of L-ascorbic acid, to measure L-ascorbic acid remaining in the tissues. Half-life of L-ascorbic acid in tissues was found to be approximately 4 days.

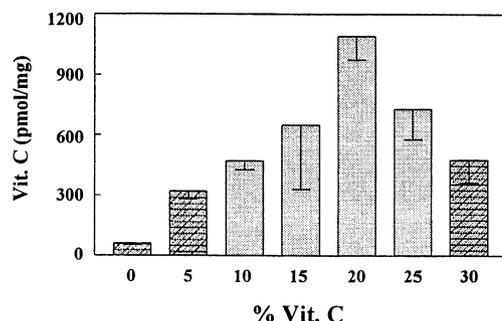


Figure 2. Effect of concentration on percutaneous absorption. Varying concentrations of L-ascorbic acid pH 3.2 were applied to pig skin for 24 hours. Skin levels of L-ascorbic acid are expressed as mean \pm SD ($n = 3$).

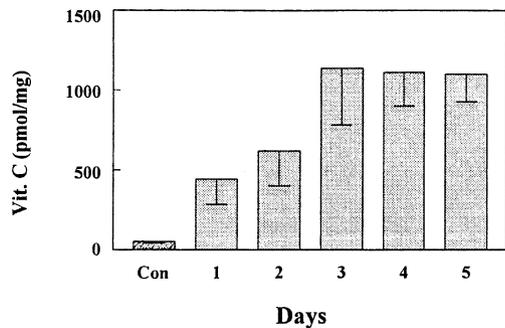


Figure 3. Time course of percutaneous absorption. 15% L-ascorbic acid pH 3.2 was applied daily to pig skin for varying times. Skin levels of L-ascorbic acid are expressed as mean \pm SD ($n = 3$).

Ascorbic Acid Derivatives

Because L-ascorbic acid is an unstable molecule to formulate for topical use, more stable derivatives of L-ascorbic acid have been utilized in topical formulations. Although esters of ascorbic acid are more stable and readily converted to L-ascorbic acid after oral ingestion, it is not clear that derivatives, after topical application, are absorbed into the skin or converted to L-ascorbic acid after penetration. We have tested commercially available high concentration formulations of magnesium ascorbyl phosphate and ascorbyl-6-palmitate to see if topical application resulted in elevated skin levels of L-ascorbic acid (Figure 5). Neither ester significantly increased L-ascorbic acid skin levels.

Dehydroascorbic Acid

Since dehydroascorbic acid can be enzymatically converted to L-ascorbic acid in the body, we asked whether topical dehydroascorbic acid could preferentially raise skin L-ascorbic acid levels. Neither 20 mM nor 1 M solutions of dehydroascorbic acid were effective. Skin levels of L-ascorbic acid were 7.51 ± 3.34

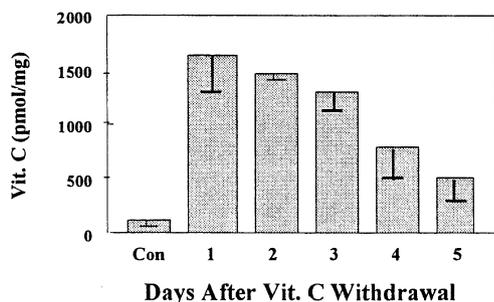


Figure 4. Washout of skin L-ascorbic acid. Skin levels of L-ascorbic acid were saturated by five daily applications to pig skin of 15% L-ascorbic acid pH 3.2. After varying intervals skin levels of L-ascorbic acid were determined. Skin levels of L-ascorbic acid are expressed as mean \pm SD ($n = 3$).

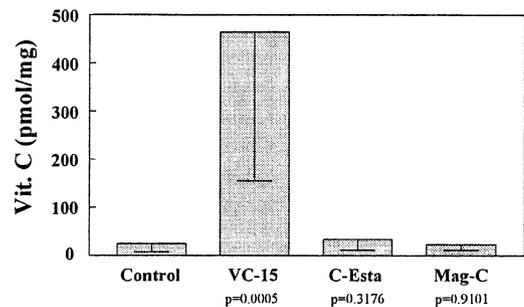


Figure 5. Percutaneous absorption of ascorbic acid derivatives. 15% L-ascorbic acid pH 3.2 (VC-15), 10% ascorbyl-6-palmitate (C-Esta), and 12% magnesium ascorbyl phosphate (Mag-C) were applied to pig skin for 24 hours. Skin levels of L-ascorbic acid are expressed as mean \pm SD ($n = 10$). *P* values are expressed versus control. The *P* value of VC-15 versus each product is also 0.0005.

pmol/mg for 20 mM dehydroascorbic acid and 8.70 ± 2.13 pmol/mg for 1 M dehydroascorbic acid ($n = 4$) and 9.24 ± 3.55 for control skin.

Discussion

L-ascorbic acid is the most plentiful antioxidant in body fluids¹⁵ and in the skin.¹² It efficiently neutralizes reactive oxygen species including superoxide anion,²⁴ hydroxyl radical,²⁵ singlet oxygen,²⁶ and peroxy-nitrite.²⁷ It is a particularly efficient antioxidant because in one electron transfer reaction, its free radical intermediate, ascorbic acid free radical, has low pro-oxidant activity²⁸ and is enzymatically regenerated back to L-ascorbic acid. Moreover, L-ascorbic acid's efficiency extends to lipophilic antioxidants as well; it regenerates oxidized vitamin E molecules.^{29,30} Maximum skin levels of L-ascorbic acid from ingestion are regulated by active transport mechanisms that preclude increasing levels by further ingestion.³¹ In this study we identify formulation characteristics that allow us to bypass these controls and increase the skin reservoir by direct topical application.

This study reveals the critical importance of formulation pH for percutaneous absorption of L-ascorbic acid. Not until the pH was 3.5 or lower were cutaneous levels increased. Since the pKa of L-ascorbic acid is 4.2, the molecule apparently must be un-ionized for delivery across the stratum corneum barrier.³² Percutaneous absorption of varying L-ascorbic acid concentrations formulated at acid pH increased steadily to a maximum of 20%. Higher concentrations were less effective for unknown reasons. Daily application for 3 days of 15% L-ascorbic acid formulated at pH 3.2 resulted in saturating skin concentrations of L-ascorbic acid at more than 20 times control values. After saturating the skin reservoir, the L-ascorbic acid was ap-

parently stabilized and remained in the tissue with a half-life approaching 4 days. We have no data about the relative distribution of ascorbic acid in the skin. A persistent reservoir of antioxidant provides an important and attractive photoprotection strategy when contrasted to sunscreens which must be applied daily.

In the body both L-ascorbic acid and dehydroascorbic acid can be transported into cells, the latter converted efficiently into L-ascorbic acid by glutathione and enzymatic reduction.³³ L-ascorbic acid requires a specific protein to be transported into cells. Hexose transporters transport dehydroascorbic acid. Indeed dehydroascorbic acid is preferentially accumulated in comparison to L-ascorbic acid in HaCaT, a human keratinocyte cell line.³⁴ Our experiments to determine whether dehydroascorbic acid was preferable to L-ascorbic acid for topical use failed to reveal any increase in skin levels of L-ascorbic acid with dehydroascorbic acid.

Topical magnesium ascorbyl phosphate and ascorbyl-6-palmitate in the tested formulations failed to increase skin levels of L-ascorbic acid. Previous studies have documented the marginal percutaneous absorption of magnesium ascorbyl phosphate;³⁵ as a charged molecule, it would not be expected to traverse the stratum corneum. Previous studies of ascorbyl-6-palmitate failed to demonstrate protection against photoaging in mouse skin;³⁶ in comparison, L-ascorbic acid was protective even though the formulation used was not optimal for percutaneous delivery. Although ascorbyl-6-palmitate appears to readily enter skin,³⁶ its conversion to L-ascorbic acid may be inefficient. Ascorbyl-6-palmitate appears to remain on the extracellular surface of cells and may not be readily converted to L-ascorbic acid.³⁷ Indeed in human skin fibroblast culture 10^{-5} M L-ascorbic acid, which is the physiologic concentration in humans, stimulated cell growth, whereas similar levels of ascorbyl-6-palmitate were toxic.³⁸

Topical antioxidants have been previously demonstrated to be photoprotective for skin. Topical L-ascorbic acid has been shown to decrease UVB erythema in pig¹⁷ and human skin.³⁹ It also lessened UVA-psoralen phototoxic injury in pig skin.¹⁷ In hairless mice, topical L-ascorbic acid decreased photoaging changes in human⁴⁰ and hairless mouse skin.³⁶ In addition, it prevented UVB immunosuppression and tolerance to dinitrochlorobenzene (DNCB).¹⁸ In persons with either basal cell carcinoma or squamous cell carcinoma, serum levels of L-ascorbic acid were below control levels.⁴¹ Topical α -tocopherol decreased photoinjury in skin^{36,42-47} and prevented UV immunosuppression.^{45,48,49} Herbal antioxidants, including silymarin, a flavonoid present in the thistle plant,⁵⁰ and grape seed polyphenols⁵¹ have been shown to prevent UV-induced squamous cell skin cancer in mice.

Although all UV light can produce oxidative stress in skin, UVA is more efficient. The peak UV spectrum for generation of singlet oxygen from trans-urocanic acid, a known photoreceptor in skin, is about 350 nm.⁵² The UV spectrum is similar to that previously demonstrated to generate photoaging changes in mouse skin.⁵³ UVA from artificial light sources has been demonstrated to generate photoaging changes in sun-protected skin.^{54,55} Similar changes have been demonstrated using only long-wave UVA (340–400 nm).⁵⁶ Studies in skin cells,^{57,58} as well as human skin,¹⁰ implicate activation of matrix metalloproteinase by a mechanism involving singlet oxygen, AP-1, and NF- κ B. In preliminary studies, antioxidants reverse activation of AP-1.^{58,59} Previous studies have demonstrated that photoaging changes are even more pronounced in smokers than sunbathers, and the combination of smoking and sun exposure was most damaging of all.⁶⁰ Presumably smoking and UV exposure are both damaging to skin by generating reactive oxygen species. In smokers, serum ascorbic acid levels were reduced;⁶¹ they required an elevated minimum daily dose of L-ascorbic acid to keep body stores saturated.²⁶

In addition to its antioxidant effects, L-ascorbic acid is important for wound healing.^{62,63} It is essential for collagen synthesis; in addition to its cofactor requirements for lysyl hydroxylase and prolyl hydroxylase,⁶⁴ it stimulates transcription of collagen genes.⁶⁵ It has been used as a skin lightener; it inhibits tyrosinase.⁶⁶ Topical L-ascorbic acid has been reported to be useful for healing of skin resurfaced by CO₂ laser; it reduced postlaser erythema.⁶⁷ Topical L-ascorbic acid together with 20% glycolic acid used for 3 months improved striae alba.⁶⁸

Topical L-ascorbic acid provides a safe and effective supplement to normal tissue stores to enhance photoprotection, improve wound healing, and increase antioxidant defenses. Details of formulation are essential if it is to be maximally effective. It must be formulated at high concentration and at a pH lower than 3.5 to be effective. After being delivered into the skin, L-ascorbic acid is stabilized and remains in the tissue for a period of days. Magnesium ascorbyl phosphate and ascorbyl-6-palmitate are not effective substitutes for L-ascorbic acid in topical formulations. Although they are effective vitamin C derivatives for oral use, they are apparently ineffective for increasing tissue vitamin C levels when applied to the skin.

References

1. Black HS, deGrujil FR, Forbes PD, Cleaver JE. Photocarcinogenesis: an overview. *Photochem Photobiol* 1997;40:29–47.
2. de Laet JM, de Grujil FR. The role of UVA in the aetiology of non-melanoma skin cancer. *Cancer Surv* 1996;26:173–91.

3. Stary A, Robert C, Sarasin A. Deleterious effects of ultraviolet A radiation in human cells. *Mutat Res* 1997;383:1-8.
4. Tyrrell RM. UVA (320-380 nm) radiation as an oxidative stress. In: *Oxidative stress, oxidants and antioxidants*. New York: Academic Press, 1991:57-83.
5. Tyrrell RM. Oxidant, antioxidant status and photocarcinogenesis: the role of gene activation. *Photochem Photobiol* 1996;63:380-86.
6. Ahmed NU, Ueda M, Nikaido O, Osawa T, Ichihashi M. High levels of 8-hydroxy-2'-deoxyguanosine appear in normal human epidermis after a single dose of ultraviolet radiation. *Br J Dermatol* 1999;140:226-31.
7. Wenzel E, Pool S, Timmerman AJ, van der Schans GP, Roza L, Schothorst AA. Physiological doses of ultraviolet irradiation induce DNA strand breaks in cultured human melanocytes, as detected by means of an immunochemical assay. *Photochem Photobiol* 1997;66:828-30.
8. Parsons PG, Hayward IP. Inhibition of DNA repair synthesis by sunlight. *Photochem Photobiol* 1985;42:287-93.
9. Fisher GJ, Datta SC, Talwar HS, et al. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature* 1996;379:335-9.
10. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997;337:1419-29.
11. Runger TM. Role of UVA in the pathogenesis of melanoma and non-melanoma skin cancer—a short review. *Photodermatol Photoimmunol Photomed* 1999;6:212-6.
12. Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol* 1994;102:122-4.
13. Nishikimi M, Fukuyama R, Minoshima S, Shimizu N, Yagi K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulonogamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J Biol Chem* 1994;269:13685-8.
14. Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* 1996;93:3704-9.
15. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 1989;86:6377-81.
16. Steenvoorden DPT, Beijersbergen van Henegouwen GMJ. The use of endogenous antioxidants to improve photoprotection. *Photochem Photobiol* 1997;41:1-10.
17. Darr D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 1992;127:247-53.
18. Nakamura T, Pinnell SR, Darr D, et al. Vitamin C abrogates the deleterious effects of UVB radiation on cutaneous immunity by a mechanism that does not depend on TNF- α . *J Invest Dermatol* 1997;109:20-24.
19. Washko PW, Wang Y, Levine M. Ascorbic acid recycling in human neutrophils. *J Biol Chem* 1993;268:15531-5.
20. Monteiro-Riviere N, Riviere J. The pig as a model for cutaneous pharmacology and toxicology research. In: *Tumbleson M, Schook L, eds. Swine in biomedical research*. New York: Plenum, 1996:425-58.
21. Monteiro-Riviere N, Van Miller J, Simon G, Joiner R, Brooks J, Riviere J. Comparative in vitro dermal absorption of nonylphenol and nonylphenol ethoxylates (NPE-4 and NPE-9) through human, porcine and rat skin. *Toxicol Ind Health* 2000;16:49-57.
22. Washko PW, Hartzell WO, Levine M. Ascorbic acid analysis using high-performance liquid chromatography with coulometric electrochemical detection. *Anal Biochem* 1989;181:276-82.
23. Dhariwal KR, Hartzell WO, Levine M. Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. *Am J Clin Nutr* 1991;54:712-6.
24. Scarpa M, Stevanato R, Viglino P, Rigo A. Superoxide ion as active intermediate in the autooxidation of ascorbate by molecular oxygen. Effect of superoxide dismutase. *J Biol Chem* 1983;258:6695-7.
25. Cabelli DE, Bielski BH. Kinetics and mechanism for the oxidation of ascorbic acid/ascorbate by HO₂/O₂-radicals. A pulse radiolysis and stopped-flow photolysis study. *J Phys Chem* 1983;87:1809-12.
26. Bodannes RS, Chan PC. Ascorbic acid as a scavenger of singlet oxygen. *FEBS Lett* 1979;105:195-6.
27. Squadrito GL, Jin X, Pryor WA. Stopped-flow kinetic study of the reaction of ascorbic acid with peroxynitrite. *Arch Biochem Biophys* 1995;322:53-9.
28. Buettner GR, Jurkiewicz BA. Chemistry and biochemistry of ascorbic acid. In: *Cadenas E, Packer L, eds. Handbook of antioxidants*. New York: Marcel Dekker, 1996:91-115.
29. Halpner AD, Handelman GJ, Harris JM, Belmont CA, Blumberg JB. Protection by vitamin C of loss of vitamin E in cultured rat hepatocytes. *Arch Biochem Biophys* 1998;359:305-9.
30. Waters RE, White LL, May JM. Liposomes containing alpha-tocopherol and ascorbate are protected from an external oxidant stress. *Free Radic Res* 1997;26:373-9.
31. Rumsey SC, Levine M. Absorption, transport, and disposition of ascorbic acid in humans. *J Nutr Biochem* 1998;9:116-30.
32. Redelmeier TESH. Pharmacokinetics and topical applications of drugs. In: *Freedberg I, ed. Fitzpatrick's dermatology in general medicine*. New York: McGraw-Hill, 1999:2699-707.
33. Rumsey SC, Wang Y, Levine M. Vitamin C. In: *Papas AM, ed. Antioxidant status, diet, nutrition, and health*. Boca Raton: CRC Press, 1999:159-88.
34. Savini I, Duflo S, Avigliano L. Dehydroascorbic acid uptake in a human keratinocyte cell line (HaCaT) is glutathione-independent. *Biochem J* 2000;345(pt 3):665-72.
35. Kameyama K, Sakai C, Kondoh S, et al. Inhibitory effect of magnesium L-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. *J Am Acad Dermatol* 1996;34:29-33.
36. Bissett DL, Chatterjee R, Hannon DP. Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol Photoimmunol Photomed* 1990;7:56-62.
37. Ross D, Mendiratta S, Qu ZC, Cobb CE, May JM. Ascorbate 6-palmitate protects human erythrocytes from oxidative damage. *Free Radic Biol Med* 1999;26:81-9.
38. Perricone NV. Topical vitamin C ester (ascorbyl palmitate). *J Geriatr Dermatol* 1997;5:162-70.
39. Murray J, Darr D, Reich J, Pinnell S. Topical vitamin C treatment reduces ultraviolet B radiation-induced erythema in human skin. *J Invest Dermatol* 1991;96:587.
40. Traikovich SS. Use of topical ascorbic acid and its effects on photo-damaged skin topography. *Arch Otolaryngol Head Neck Surg* 1999;125:1091-8.
41. Vural P, Canbaz M, Selcuki D. Plasma antioxidant defense in actinic keratosis and basal cell carcinoma. *J Eur Acad Dermatol Venereol* 1999;113:96-101.
42. Darr D, Dunston S, Kamino H, Faust H, Pinnell SR. Effectiveness of a combination of vitamin C and E in inhibiting UV damaged to porcine skin. *J Invest Dermatol* 1993;101:471.
43. Khettab N, Amory MC, Briand G, et al. Photoprotective effect of vitamins A and E on polyamine and oxygenated free radical metabolism in hairless mouse epidermis. *Biochimie* 1999;70:1709-13.
44. Lopez-Torres M, Thiele JJ, Shindo Y, Han D, Packer L. Topical application of α -tocopherol modulates the antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. *Br J Dermatol* 1998;138:207-15.
45. Gensler HL, Magdaleno M. Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by UV irradiation. *Nutr Cancer* 1991;15:97-110.
46. Potapenko AY, Abijev GA, Pistov MY, et al. PUVA-induced erythema and changes in mechano-electrical properties of skin. Inhibition by tocopherols. *Arch Dermatol Res* 1984;276:12-8.
47. McVean M, Liebler DC. Inhibition of UVB induced DNA photo-damage in mouse epidermis by topically applied alpha-tocopherol. *Carcinogenesis* 1997;18:1617-22.
48. Steenvoorden DP, Beijersbergen VH. Protection against UV-induced systemic immunosuppression in mice by a single topical application of the antioxidant vitamins C and E. *Int J Radiat Biol* 1999;75:747-55.
49. Yuen KS, Halliday GM. α -Tocopherol, an inhibitor of epidermal lipid peroxidation, prevents ultraviolet radiation from suppressing the skin immune system. *Photochem Photobiol* 1997;65:587-92.
50. Lahiri-Chatterjee M, Katiyar SK, Mohan RR, Agarwal R. A fla-

- vonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. *Cancer Res* 1999;59:622-32.
51. Zhao J, Wang J, Chen Y, Agarwal R. Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3'-gallate as the most effective antioxidant constituent. *Carcinogenesis* 1999;20:1737-45.
 52. Hanson KM, Simon JD. Epidermal trans-urocanic acid and the UV-A-induced photoaging of the skin. *Proc Natl Acad Sci USA* 1998;95:10576-8.
 53. Bissett DL, Hannon DP, Orr TV. Wavelength dependence of histological, physical, and visible changes in chronically UV-irradiated hairless mouse skin. *Photochem Photobiol* 1989;50:763-9.
 54. Lavker RM, Gerberick GF, Veres D, Irwin CJ, Kaidbey KH. Cumulative effects from repeated exposures to suberythemal doses of UVB and UVA in human skin [see comments]. *J Am Acad Dermatol* 1995;32:53-62.
 55. Lowe NJ, Meyers DP, Wieder JM, et al. Low doses of repetitive ultraviolet A induce morphologic changes in human skin. *J Invest Dermatol* 1995;105:739-43.
 56. Lavker RM, Veres DA, Irwin CJ, Kaidbey KH. Quantitative assessment of cumulative damage from repetitive exposures to suberythemal doses of UVA in human skin. *Photochem Photobiol* 1995;62:348-52.
 57. Wlaschek M, Wenk J, Brenneisen P, et al. Singlet oxygen is an early intermediate in cytokine-dependent ultraviolet-A induction of interstitial collagenase in human dermal fibroblasts *in vitro*. *FEBS Lett* 1997;413:239-42.
 58. Djavaheri-Mergny M, Mergny JL, Bertrand F, et al. Ultraviolet-A induces activation of AP-1 in cultured human keratinocytes. *FEBS Lett* 1996;384:92-6.
 59. Kang S, Fisher G, Duell E, Wan Y, Varani J, Voorhees J. The antioxidant N-acetyl cysteine increases the content of the endogenous antioxidant glutathione and prevents UV-induction of matrix metalloproteinases in human skin *in vivo*. *J Invest Dermatol* 1999;112:658.
 60. Kadunce DP, Burr R, Gress R, Kanner R, Lyon JL, Zone JJ. Cigarette smoking: risk factor for premature facial wrinkling. *Ann Intern Med* 1991;114:840-44.
 61. Lykkesfeldt J, Loft S, Nielsen JB, Poulsen HE. Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking. *Am J Clin Nutr* 1997;65:959-63.
 62. Vaxman F, Olender S, Lambert A, Nisand G, Grenier JF. Can the wound healing process be improved by vitamin supplementation? Experimental study on humans. *Eur Surg Res* 1996;28:306-14.
 63. Taylor TV, Rimmer S, Day B, Butcher J, Dymock IW. Ascorbic acid supplementation in the treatment of pressure-sores. *Lancet* 1974;2:544-6.
 64. Kivirikko KI, Myllyla R. Post-translational processing of procollagens. *Ann N Y Acad Sci* 1985;460:187-201.
 65. Tajima S, Pinnell SR. Ascorbic acid preferentially enhances type I and III collagen gene transcription in human skin fibroblasts. *J Dermatol Sci* 1996;11:250-53.
 66. Maeda K, Fukuda M. Arbutin: mechanism of its depigmenting action in human melanocyte culture. *J Pharmacol Exp Ther* 1996;276:765-9.
 67. Alster T, West TB. Effect of topical vitamin C on postoperative carbon dioxide laser resurfacing erythema. *Dermatol Surg* 1998;24:331-4.
 68. Ash K, Lord J, Zukowski M, McDaniel DH. Comparison of topical therapy for striae alba (20% glycolic acid/10% L-ascorbic acid). *Dermatol Surg* 1998;24:849-56.