Cutaneous photodamage, oxidative stress, and topical antioxidant protection

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New methods to protect skin from photodamage from sun exposure are necessary if we are to conquer skin cancer and photoaging. Sunscreens are useful, but their protection is not ideal because of inadequate use, incomplete spectral protection, and toxicity. Skin naturally uses antioxidants (AOs) to protect itself from photodamage. This scientific review summarizes what is known about how photodamage occurs; why sunscreens—the current gold standard of photoprotection—are inadequate; and how topical AOs help protect against skin cancer and photoaging changes. This review is intended to be a reference source, including pertinent comprehensive reviews whenever available. Although not all AOs are included, an attempt has been made to select those AOs for which sufficient information is available to document their potential topical uses and benefits. Reviewed are the following physiologic and plant AOs: vitamin C, vitamin E, selenium, zinc, silymarin, soy isoflavones, and tea polyphenols. Their topical use may favorably supplement sunscreen protection and provide additional anticarcinogenic protection. (J Am Acad Dermatol 2003;48:1-19.)

Learning objective: At the completion of this learning activity, participants should have an understanding of current information about how the sun damages skin to produce skin cancer and photoaging changes, how the skin naturally protects itself from the sun, the shortcomings of sunscreens, and the added advantages of topical AOs for photoprotection.

PHOTODAMAGE

Sunlight coupled with living in an oxygen-rich atmosphere causes unwanted and deleterious stresses on skin. The most severe consequence of photodamage is skin cancer. Less severe photoaging changes result in wrinkling, scaling, dryness, and mottled pigment abnormalities consisting of hyperpigmentation and hypopigmentation. For a photochemical reaction to occur in the skin, ultraviolet (UV) light from the sun must be absorbed by a chromophore, beginning a series of photochemic reactions that may result in skin cancer or photoaging changes.[1] These photochemical reactions can result in changes to DNA, including oxidation of nucleic acids. Oxidative reactions can also modify proteins and lipids, resulting in changes in function. Their accumulation may result in tissue aging. The body is well equipped to deal with oxidative stress, naturally using antioxidant (AO) enzymes and nonenzymic AOs to lessen these changes. However, sunlight and other free-radical generators (eg, smoking, pollution) can overwhelm the system, making natural protective controls inadequate, resulting in oxidative damage.

Abbreviations used:

AO:	antioxidant
AP-1:	activation protein-1
DMBA:	7,12-dimethyl benzanthracene
ERα:	estrogen receptor α
ERβ:	estrogen receptor β
LDL:	low-density lipoprotein
MED:	minimal erythema dose
MMP:	matrix metalloproteinase
NADPH:	nicotinamide adenine dinucleotide phosphate reduced
NF-κB:	nuclear factor-κ B
PKCδ:	phosphokinase Cδ
ROS:	reactive oxygen species
SPF:	sun protection factor
TPA:	12-*O*-tetradecanoylphorbol-13-acetate
UV:	ultraviolet
VEGF:	vascular endothelial growth factor

Chromophores

Many candidates for substances capable of absorbing UV light in skin exist, but DNA and urocanic

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acid have been identified as being biologically important.

DNA may absorb UVB (290-320 nm), directly inducing changes between adjacent pyrimidine bases on one strand of DNA. Cyclopyrimidine dimers, particularly thymine dimers or, less commonly, (6-4)-photoproducts, may be generated. The action spectrum for these changes is maximal at about 300 nm, although UVA (320-400 nm) can also generate thymine dimers.[2,3] These DNA changes are constantly being repaired by nucleotide excision repair[4]; the photoproduct recognition proteins are those defective in xeroderma pigmentosum. Whenever repair is incomplete, signature C→T and CC→TT mutations characteristic for UV photodamage may result. If damage to the genome is great, p53 and its associated proteins will induce apoptosis of the irradiated keratinocyte. p53 is induced by UVB, perhaps as a response to excised thymine dimers.[5] If the UV signature mutations occur in p53, quality control over the genome may be lost. Clonal expansion of these photomodified keratinocytes may give rise to an actinic keratosis.[6] If the second p53 allele is also mutated, a squamous cell carcinoma may arise. If the signature mutations occur in *patched* or other members of the hedgehog signaling pathway, a basal cell carcinoma may occur.[7,8]

Urocanic acid has recently been identified as a second chromophore for photochemic reactions in skin.[9,10] One photon of light contains enough energy to generate singlet oxygen.[11] When UV light is absorbed by *trans*-urocanic acid, singlet oxygen is generated. The peak action spectrum for this reaction is about 345 nm. Urocanic acid occurs in skin as a by-product of filaggrin breakdown. It is found in high concentrations superficially in the epidermis. Once singlet oxygen is formed, this highly reactive oxygen species (ROS) can attack cell membranes and generate additional ROS.

Reactive oxygen species

ROS are an inherent part of the anabolism and catabolism of tissues, including skin.[11-13] Most oxygen in the body is used in cellular metabolism. Through a series of 1-electron subtractions, molecular oxygen is in sequence changed to superoxide anion, hydrogen peroxide, hydroxyl radical, and, finally, to water. Most reactions occur in mitochondria and are related to energy production. Cellular enzymes and controlled metabolic processes ordinarily keep oxidative damage to cells at a minimum. In times of increased oxidative stress, however—including high metabolic demands and outside forces such as sunlight, smoking, and pollution—protective controls may not be adequate and oxidative damage may occur. The most damage occurs from free radicals. Free radicals are defined as atoms or molecules with an unpaired electron. Examples include superoxide anion, peroxyl radical, and hydroxyl radical. These molecules are extremely chemically reactive and short-lived; they react at the place where they are created. Other reactive molecules such as molecular oxygen, singlet oxygen, and hydrogen peroxide are not free radicals per se, but are capable of initiating oxidative reactions and generating free-radical species. Together, these free radicals and reactive oxygen molecules are called ROS.

The cell is well equipped to deal with most oxidative damage.[12] Cellular integrity is maintained by enzymes, including catalase, glutathione reductase, and glutathione peroxidases, which collectively destroy hydrogen peroxide and lipid hydroperoxides. In addition, superoxide dismutase destroys superoxide. The extracellular space is protected from superoxide anion by extracellular superoxide dismutase. Nonenzymic AOs protecting skin include glutathione and ascorbic acid in the aqueous phase and vitamin E and ubiquinol-10 in the lipid phase, particularly in membranes.

Photocarcinogenesis

UVB irradiation is a complete carcinogen and can generate squamous cell carcinomas in animals.[14] As previously described (see "Chromophores" section), DNA absorbs UVB, leading to signature UV-induced DNA mutations C→T and CC→TT. The UV action spectrum for generation of squamous cell carcinoma occurs mostly in the UVB, although there is a peak of activity in the UVA (320-400 nm).[15] Whereas UVB is important for tumor initiation, UVA predominantly causes tumor promotion.[16] Compared with UVB, UVA generates more oxidative stress.[17-20] At levels found in sunlight, UVA is 10 times more efficient than UVB at causing lipid peroxidation.[21] UVA is more cytotoxic than UVB.[16] UVA damages DNA by causing strand breaks and oxidation of nucleic acids.[16,22] The characteristic mutagenic lesion generated by oxidative stress is 8-hydroxyguanine, which generates G:C to T:A transversions by pairing with adenine, instead of cytosine, during replication.[23] UVA can inhibit DNA repair.[24] In addition, UVA can induce matrix metalloproteinase (MMP) synthesis[25,26] that can augment the biologic aggressiveness of skin cancer.

Sunlight can suppress the immune function of skin and promote skin cancer formation.[27] Approximately 40% of human beings are susceptible to UV immunosuppression; however, virtually all persons with basal cell or squamous cell carcinomas demonstrate UV immunosuppression. Although most stud-

Table I. Histology of photoaging

Epidermis
A. Keratinocytes—irregular size and shape, loss of polarity
B. Melanocytes—irregular shape, pockets of increased and decreased numbers
C. Langerhans cells—decreased
Dermis
A. Collagen—basophilic staining, irregular and disorganized
B. Elastin tissue—increased, amorphous
C. Glycosaminoglycans—increased

ies of UV immunosuppression have been conducted using UVB,[28] recent studies have highlighted the importance of UVA in causing immunosuppression[29] and the ability of AOs to prevent immunosuppression.[28 30,31] The importance of immunosuppression on the biologic behavior of skin cancer is best appreciated in persons immunosuppressed for organ transplantation, with their extreme incidence and lethality of skin cancer.[32-37]

In addition to more efficiently generating ROS in skin, UVA causes additional biologic effects different from UVB. Sunlight contains about 20 times as much UVA as UVB. Whereas UVB is almost entirely absorbed in the epidermis, UVA is capable of reaching dermal layers[38,39] and even affecting circulating blood cells.[40] Window glass blocks most UVB irradiation but not UVA. This creates special problems for those who spend long hours in cars.[41] Without protection, their skin may be particularly susceptible to oxidative stress. Indeed, pilots who fly transcontinental routes at high altitudes without protection have an increased susceptibility to melanoma and other skin cancers.[42,43]

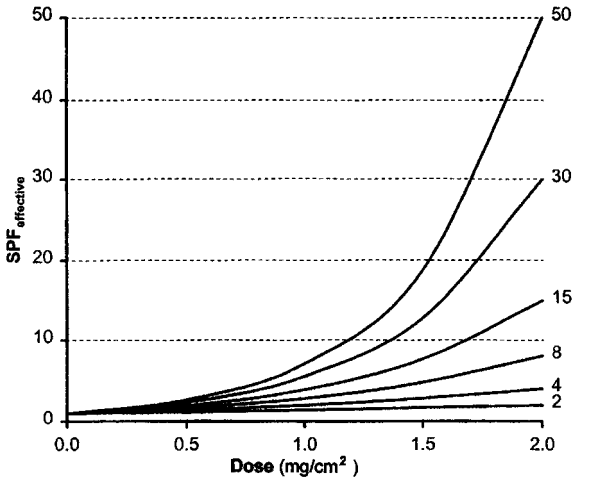
Photoaging

Sunlight exposure has a profound effect on exposed skin, producing accelerated aging changes consisting of wrinkling, dryness, telangiectasia, and pigmentary abnormalities—including lentigines as well as guttate hypermelanosis and hypomelanosis[44-46] (Table I). Histologically, the dermis is strikingly filled with an amorphous mass of deranged elastic fibers. Collagen fibers take on a basophilic hue and appear disorganized. Glycosaminoglycans are prominent. Blood vessels are dilated and tortuous. Dermal inflammatory cells are increased. Keratinocytes are irregular with a loss of polarity. Melanocytes are irregular with pockets of increased and decreased numbers. Langerhans cells are diminished in actinic skin. Because UVB is essentially completely absorbed in the epidermis, it has been important to understand that photoaging changes can be produced by UVA alone. Indeed, these changes have been produced in photoprotected skin by a small number of low-dose exposures of UVA irradiation.[47,48] Similar changes can be produced by UVA1 (340-400 nm) exposure alone.[49] Small amounts of UV irradiation result in the induction of a series of MMPs including MMP-1, MMP-2, MMP-3, and MMP-9.[50] Together these proteases are capable of degrading the collagen framework of skin. At the same time procollagen synthesis is inhibited,[51] perhaps by a mechanism related to degraded collagen.[52] Levels of procollagen I protein are decreased, whereas MMP-1 protein and MMP-2 activity are increased in exposed skin compared with unexposed skin.[53] These changes apparently occur through induction of transcription factor activation protein (AP-1) that is activated by a series of mitogen-activated protein kinases. In addition, the transcription factor, nuclear factor-κ B (NF-κB), is activated by UV irradiation, which stimulates neutrophil attraction bringing neutrophil collagenase (MMP-8) into the irradiation site to further aggravate matrix degradation. Both AP-1 and NF-κB are activated by ROS that may provide the common denominator for driving this complex biologic interaction.[54-57] Oxidative stress can also increase elastin messenger RNA levels in dermal fibroblasts providing a mechanism for the elastotic changes found in photoaged dermis.[58]

ROS can modify proteins in tissue to form carbonyl derivatives.[59] These carbonyls accumulate in the papillary dermis of photodamaged skin.[60] Lipids can also be modified by ROS. UVA can induce lipid peroxidation in membranes that can lead to altered membrane fluidity.[61]

In addition to nuclear DNA, the DNA in mitochondria can also be altered by oxidative stress.[62] Because DNA repair is less efficient in mitochondria compared with nuclei, mutations accumulate at a relatively rapid pace. A common deletion in the DNA has been identified and shown to be very common in photoaged skin when compared with sun-protected sites.[63] The deletion can be generated by UVA and is mediated by singlet oxygen.[64] These mutations may alter cell capacity to carry out oxidative phosphorylation and, in turn, may generate more oxidative stress.

Uneven hyperpigmentation and hypopigmentation is extremely common in photoaged skin. Although its cause is unclear, a recent study has demonstrated increased endothelin-1 activity in keratinocytes, and increased endothelin-B receptor and tyrosinase in solar lentigines.[65] In addition, melanogenesis can be stimulated by DNA damage. Single-stranded DNA oligonucleotides and thymine



SPF	2.0 mg/cm²	1.5 mg/cm²	1.0 mg/cm²	0.5 mg/cm²
2	2.0	1 7	1.4	1.2
4	4.0	2.8	2.0	1.4
8	8.0	4.8	2.8	1.7
15	15.0	7.6	3.9	2.0
30	30.0	12.8	5.5	2.3
50	50.0	18.8	7.1	2.7

Fig 1. Photoprotection from sunscreens. Sunscreen-use studies have demonstrated that in actual use, sunscreen application is 25% or less of that used to measure sun-protection factor (*SPF*).[69,70] SPF is not a linear relationship with concentration; therefore, at 0.5 mg/cm^2 application to skin, high SPF sunscreens provide less than SPF 3 protection. (Modified from Wulf HC, Stender IM, Lock-Andersen J. Photodermatol Photoimmun Photomed 1997;13:129-32.)

dinucleotide can stimulate pigment production in melanocytic cells associated with increased tyrosinase levels.[66]

SUNSCREENS

Sunscreens are the "gold standard" for protecting skin from photodamage.[67] Many chemicals have been developed that absorb UV light efficiently[14] and protect against erythema.[68] However, just recently, we have learned that, in actual use, sunscreens provide much less protection than expected. Sun protection factor (SPF) is measured and tested at an application to skin of 2 mg/cm^2. Controlled studies of actual sunscreen use reveal that sunscreens are applied to skin at only 0.5 mg/cm^2 or less.[69,70] SPF is not linearly proportional; thus, at an application of 0.5 mg/cm^2, no sunscreen provides more than 3-fold protection (Fig 1). Moreover, important biologic events such as DNA damage as measured by thymine dimer formation and 8-hydroxy-2′-deoxyguanosine formation,[68] as well as p53 induction and UV immunosuppression,[68,71] continue at suberythemal levels of irradiation. Sunscreens may provide a false sense of security. Finally, no sunscreen provides full spectral protection against UV light. Sunscreen ingredients may become free radicals, themselves, when activated by UV irradiation,[72] and sunscreen chemicals may be absorbed into skin[73] to potentially cause harm.

ANTIOXIDANT PROTECTION

The skin naturally relies on AOs to protect it from oxidant stress generated by sunlight and pollution.[74] A relative symphony of enzymic and nonenzymic AOs interacts to provide protection in both the intracellular and extracellular space. AO enzymes function predominantly in cells. Glutathione peroxidase and glutathione reductase reduce hydrogen peroxide and lipid hydroperoxides using glutathione. Catalase detoxifies hydrogen peroxide and is an important AO in peroxisomes. Copper-zinc superoxide dismutase and manganese superoxide dismutase protect cells from superoxide; extracellular superoxide dismutase protects the extracellular space. Enzyme activities in human skin are higher in epidermis than dermis; catalase is especially high.[75] When skin fibroblasts were irradiated with UVA, catalase activity was preferentially destroyed, superoxide dismutase activity was diminished, but glutathione peroxidase and glutathione reductase were virtually unchanged.[76] Similar results were seen when murine skin was irradiated with solar irradiation.[76]

Low-molecular-weight, nonenzymic AOs include L-ascorbic acid in the fluid phase, glutathione in the cellular compartment, vitamin E in membranes, and ubiquinol in mitochondria (Table II). On a molar basis, L-ascorbic acid is the predominant AO in skin; its concentration is 15-fold greater than glutathione, 200-fold greater than vitamin E, and 1000-fold greater than ubiquinol/ubiquinone.[75] Concentrations of AOs are higher in epidermis than dermis; 6-fold for L-ascorbic acid and glutathione, and 2-fold for vitamin E and ubiquinol/ubiquinone. Solar-simulated irradiation of murine skin reduced levels of nonenzymic AOs. Ubiquinol/ubiquinone and glutathione were most sensitive; α-tocopherol and L-ascorbic acid were less sensitive.[77] Patients with actinic keratosis and basal cell carcinoma have significantly decreased plasma levels of ascorbic acid, α-tocopherol, and glutathione.[78]

Low-molecular-weight AOs work in tissues as a coordinated interactive group of chemicals related to chemical structure, position in the tissue, and relative redox potential (Fig 2).[79] Thus, when a ROS is generated in a lipophilic structure and is reduced by α-tocopherol, the oxidized tocopherol can be

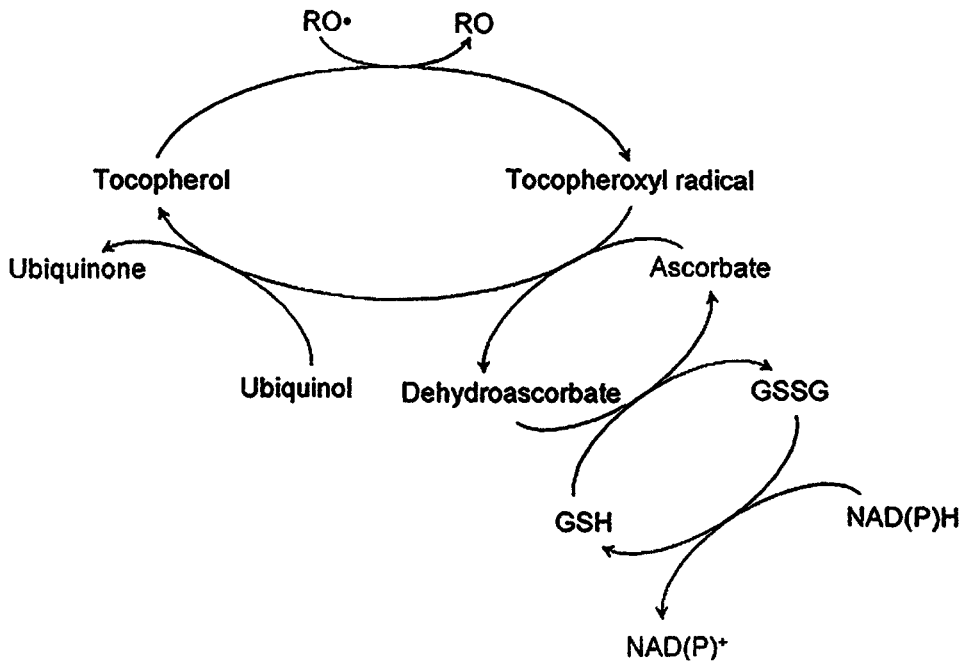


Fig 2. Interacting network of nonenzymic antioxidants. When a reactive oxygen species attacks membrane structure, it can be reduced by tocopherol that, in turn, can be regenerated by ubiquinol or ascorbic acid. Reduced ascorbic acid can be regenerated by glutathione that, in turn, can be reduced by nicotinamide adenine dinucleotide phosphate reduced (*NAD[P]H*) pool. *GSH*, Glutathione; *GSSG*, oxidized glutathione; *NAD(P)+*, nicotinamide adenine dinucleotide phosphate—oxidized form; *NAD(P)H*, nicotinamide adenine dinucleotide phosphate—reduced form; *RO°*, reactive oxygen free radical; *RO*, reduced reactive oxygen free radical. (From Podda M, Grundmann-Kollmann M. Clin Exp Dermatol 2001;26:578-82. Reproduced with permission.)

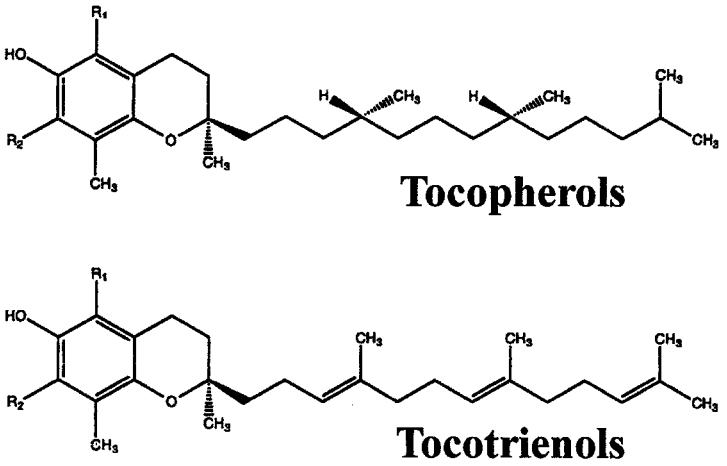
regenerated by ubiquinol or L-ascorbic acid. In turn, dehydroascorbate can be reduced by glutathione, which, in turn, can be reduced by the nicotinamide adenine dinucleotide phosphate pool. This balance may be essential for function and the system could potentially fail when any step in the process becomes rate limiting.

TOPICAL ANTIOXIDANTS

Because low-molecular-weight AOs protect skin against oxidative stress, undergoing depletion in the process, it should be desirable to add to the skin reservoir by applying the AOs directly to skin. Although AOs can be supplied to skin through diet and oral supplementation, physiologic processes related to absorption, solubility, and transport limit the amount that can be delivered into skin. Direct application has the added advantage of targeting the AOs to the area of skin needing the protection. For topical application of AOs to be useful, however, several obstacles must be overcome. AOs are inherently unstable compounds; this allows them to function in redox reactions. Instability makes them difficult to formulate in an acceptable, stable composition for cosmetic use. In addition, many AOs are deeply colored, adding to the complexity of producing an acceptable aesthetic product. To protect deeper layers of skin, AOs need to be formulated in a way that delivers them into skin. Concentrations need to be substantial and optimized to maximize skin levels. Finally, AOs need to have photoprotective effects including reduction of erythema, reduction of sunburn cell formation, reduction of DNA changes such as thymine dimers or oxidized nucleotides, reduction of UV immunosuppression, reduction of pigment abnormalities, and, eventually, reduction of skin cancer and photoaging changes.

Physiologic antioxidants

Perhaps the most obvious candidates for topical AO protection are those naturally used by the body for photoprotection. Those include vitamin C, vitamin E, ubiquinol, and glutathione. However, glutathione is a tripeptide and its ionic charges would make it an unlikely candidate for substantial percutaneous absorption.



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

Fig 3. Vitamin E structures. Molecular structures of 4 tocopherols and 4 tocotrienols comprising vitamin E. Substitution of methyl groups (CH_3) at positions R_1 and R_2 determine whether the molecules are α, β, χ, or δ.

Table II. Physiologic antioxidants

Antioxidant	Source	Distribution	Concentration (nmol/g skin)	
			Epidermis	Dermis
Vitamin C	Diet	Aqueous phase	7600.0 ± 2498.0	1311.0 ± 559.0
Glutathione	Synthesized	Cytoplasm	484.3 ± 81.4	84.8 ± 11.5
Vitamin E	Diet	Membranes, lipids	34.2 ± 4.6	18.0 ± 1.1
Ubiquinol/ubiquinone	Synthesized	Mitochondria	7.7 ± 0.5	3.2 ± 0.5

Data from Shindo Y, Witt E, Han D, Epstein W, Packer L. J Invest Dermatol 1994;102:122-4.

Vitamin C. Vitamin C (L-ascorbic acid) is the body's major aqueous phase reductant.[80,81] It is a highly water-soluble, sugar-like, low-molecular-weight α-ketolactone. By a stepwise donation of an electron, the resulting ascorbate free radical that is formed is more stable than other free radicals and can serve as a free-radical scavenger. After loss of a second electron, the resulting oxidation product, dehydroascorbic acid, can be regenerated by dehydroascorbic acid reductase, or as frequently happens, may decay as the lactone ring irreversibly opens. In addition to its AO properties, L-ascorbic acid is essential for collagen biosynthesis; it serves as a cofactor for prolyl and lysyl hydroxylases, enzymes necessary for molecular stability and intermolecular cross-linking, respectively.[82] In addition, it is important in transcriptional regulation of collagen synthesis.[83] L-ascorbic acid may inhibit elastin biosynthesis[84] and could, therefore, be useful for reducing the increased elastin accumulation that occurs in photoaged skin.[50] L-ascorbic acid reduces pigment synthesis in skin by inhibiting tyrosinase.[85] L-ascorbic acid improves epidermal barrier function,[86-88] apparently by stimulating sphingolipid production.[89]

Virtually all plants and animals synthesize L-ascorbic acid. Human beings are an exception. They have lost that ability as a result of a loss of function mutation in L-gulono-χ-lactone oxidase.[90] Human beings must get their L-ascorbic acid through diet. Even with massive supplementation, biologic control mechanisms limit the amount that can be absorbed and, subsequently, delivered into skin.[91] Topical application of L-ascorbic acid is the only way to further increase skin concentrations. Delivery of L-ascorbic acid into skin depends on removing the ionic charge on the molecule.[92] Protonation is achieved at pH below 3.5. When thus formulated, skin levels are maximized after 3 days of application of a 15% solution.[92] Once in the skin, the molecule apparently stabilizes; disappearance occurs with a half-life of approximately 4 days.

Topical L-ascorbic acid protected porcine skin from UVB- and UVA-phototoxic injury as measured by erythema and sunburn cell formation.[93] Topical L-ascorbic acid protected against UVB-induced immunosuppression and systemic tolerance to contact allergens in mice.[31] In human skin, topical L-ascorbic acid slightly enhanced levels of messenger RNA for procollagens I and III; it also enhanced levels of

procollagen processing enzymes, procollagen-N-protease, procollagen-C-protease, and lysyl oxidase in human skin.[94] Although the results are intriguing, it is not certain that the method used is sufficient to detect the small changes reported.

Derivatives of L-ascorbic acid have been substituted for L-ascorbic acid in topical formulations to improve stability. The most common of these, magnesium ascorbyl phosphate and ascorbyl-6-palmitate are readily converted to L-ascorbic acid in cell and organ culture[95] or after ingestion, but do not efficiently increase skin levels of L-ascorbic acid after topical application.[92] Magnesium ascorbyl phosphate had a skin lightening effect in an open human study as determined by chromameter measurements. The duration of use and time of year were not designated. In the same study, percutaneous absorption was only 0.09% to 0.51% of the applied dose. Intraperitoneal magnesium ascorbyl phosphate delayed skin tumor formation in UVB-irradiated hairless mice.[96] Skin levels of ascorbic acid were increased consistent with tissue conversion of the derivative. Studies in hairless mice revealed percutaneous absorption of ascorbyl-6-palmitate, but little effectiveness in an UVB-photoaging model.[97]

Vitamin E. Vitamin E is the body's major lipid phase AO.[98,99] It consists of 8 molecular forms, 4 tocopherols, and 4 tocotrienols (Fig 3). The molecules consist of a hydrophobic prenyl tail that inserts into membranes and a polar chromanol head group exposed to the membrane surface. Tocopherols and tocotrienols differ only in their prenyl tails. Tocopherols have linear, saturated tails whereas tocotrienols have a nonlinear unsaturated tail. The chromanol head of each is identical with α-, β-, χ- and δ-isomers, each containing an essential hydroxyl group, necessary for AO activity, and methyl groups varying in number and position. Although all of these isomers are available in dietary sources, human beings use predominantly α-tocopherol because a specific α-tocopherol transfer protein selectively transfers α-tocopherol into lipoproteins.[100] The major AO function of vitamin E is to prevent lipid peroxidation. When an ROS attacks membrane lipids, a peroxyl radical may form that can create more peroxyl radicals, resulting in a chain reaction that may threaten the structural integrity of the membrane.[99] Tocopherols and tocotrienols scavenge the peroxyl radical, ending the chain reaction. Vitamin E may also quench singlet oxygen.[101] Once oxidized, vitamin E can be regenerated back to its reduced form by L-ascorbic acid, allowing it to be reactivated without creating a new membrane structure[98] (Fig 2). The relative AO activities of tocopherol in lipid systems is $\alpha > \beta > \chi > \delta$.[99] Tocotrienols may have greater AO activities in lipid structures than tocopherols.[98] Vitamin E measurements in mouse tissues revealed substantial enrichment of tocotrienols in skin compared with other tissues.[102] Vitamin E is especially abundant in stratum corneum, delivered there in sebum.[102,103] Its concentration is highest at the lower levels of the stratum corneum, with a decreasing gradient outward. The stratum corneum is the outermost defense of the body and first to absorb the oxidative stress of sunlight and pollution. Vitamin E is depleted in the process and, in the absence of co-AOs, is unable to be regenerated. Vitamin E is important for protecting the lipid structures of the stratum corneum and for protecting stratum corneum proteins from oxidation. The lipophilic nature of vitamin E makes it attractive for application to and absorption into skin.[104] Several studies have documented photoprotective effects when vitamin E was topically applied to animal skin. Topical α-tocopherol protected rabbit skin against UV-induced erythema,[105] mouse skin against UV-induced lipid peroxidation,[106] mice against UV-induced photoaging changes,[97,107] mice against UV immunosuppression,[108-110] and mice against UV photocarcinogenesis.[109,111] Follow-up studies to investigate the mechanism of inhibition of photocarcinogenesis have revealed that α-tocopherol inhibited UV-induced cyclopyrimidine dimer formation in mouse skin in the epidermal *P53* gene.[112] In addition to its photoprotective effects, α-tocopherol inhibits melanogenesis; it inhibited melanin formation in human melanoma cells and demonstrated inhibitory activity against tyrosinase and tyrosine.[113] It should be noted that α-tocopherol has modest UV absorption near 290 nm and that some of its topical photoprotective effects may be related.[114]

Esterification of the hydroxyl group on the chromanol ring helps stabilize α-tocopherol in topical formulations. Because this hydroxyl group is essential for AO activity, the ester must be hydrolyzed before there is biologic activity. This reaction readily occurs after oral ingestion or in cell or organ culture studies, but appears to be very slow after topical application to skin. In human studies, α-tocopheryl acetate was substantially absorbed into skin, but was not metabolized to free α-tocopherol.[115] In mouse studies, topical α-tocopheryl succinate and α-tocopheryl acetate not only failed to inhibit UVB-induced immunosuppression and carcinogenesis, but actually appeared to enhance carcinogenesis.[116] Topical α-tocopheryl acetate was less effective than α-tocopherol against UV-induced erythema in rabbits,[105] UV-induced photoaging in mice,[97] and UV-induced free-radical formation in mice.[107] Topical α-tocopheryl succinate also was less effective than

α-tocopherol in protecting against UV-induced blistering, tanning, and skin cancer in mice.[110]

Combination vitamin C and vitamin E. Substantial experimental evidence reveals an interacting dependence of vitamins C and E in AO defense. In experimental lipid membrane[117-119] and cellular systems,[120,121] vitamin C protects vitamin E from oxidation. Vitamin E in membranes ends chain reactions produced by peroxyl radicals and is oxidized in the process. Because the redox potential of vitamin C is below that of vitamin E, it is capable of reducing oxidized vitamin E and regenerating its activity without replacing it in the membrane.[122] Oral combination vitamins C and E in high doses provide protection against UV-induced erythema in human beings,[123,124] whereas either vitamin alone is ineffective.[124] The topical combination of 15% L-ascorbic acid and 1% α-tocopherol provided 4-fold protection against UV-induced erythema and thymine dimer formation in porcine skin.[125] In combination with melatonin, vitamins C and E protect human skin from UV-induced erythema.[126] Topical combination vitamins C and E inhibit UV-induced tanning and immunosuppression in mice[127] and tanning in human beings.[128]

Selenium. Selenium is an essential micronutrient required for at least 2 types of enzymes involved in defense against oxidative stress in mammals.[129-132] These enzymes, glutathione peroxidase and thioredoxin reductase, represent a significant portion of the cell's total defense against oxidative stress and are vital to maintaining a stable redox balance in the cell. In selenoenzymes, the selenium is present as selenocysteine, and a specific and elaborate system exists for its incorporation into these proteins.[133] The activity of selenoenzymes can be increased by selenium supplementation.[134-136] Several cellular studies have demonstrated the protective effects of selenium for UV-induced damage including cytotoxicity,[137-140] DNA oxidation,[141] DNA damage,[129] interleukin 10 expression,[142] and lipid peroxidation.[143] Oral sodium selenite protected hairless mice against UV-induced erythema and subsequent pigmentation.[144] Oral selenium protected mice against UV-induced skin cancer,[145 146] although an oral trial in human beings failed to protect against basal or squamous cell carcinoma.[147] Topical L-selenomethionine protected mice against UV-induced erythema and skin cancer.[148] In human beings, topical L-selenomethionine increased the minimal erythema dose in a dose-responsive fashion.[149]

Zinc. Zinc is an essential human element. Skin and appendages are rich in zinc, containing approximately 20% of the body's total.[150] Zinc binds to a number of biologic molecules and influences their conformation, stability, and activity. Zinc serves as a catalyst for enzymes responsible for DNA replication, gene transcription, and RNA and protein synthesis.[151,152] Zinc has an important AO effect in tissues.[153] Two different AO mechanisms have been proposed. Zinc may replace potentially damaging redox-active molecules, such as iron and copper, at critical sites in cell membranes and proteins. Alternatively, zinc may induce the synthesis of metallothionein, sulfhydryl-rich proteins that neutralize free radicals.

In cellular studies using human skin fibroblasts, zinc protected against UV-induced cytotoxicity,[138] DNA damage,[129,154] and lipid peroxidation.[138,155] Oral zinc supplementation reduced UV immunosuppression to contact hypersensitivity in mice.[150] When similar studies were conducted in transgenic mice with null mutations in metallothionein-I and metallothionein-II genes, UV immunosuppression was not altered by zinc. These studies suggest that zinc induction of metallothionein in skin protected against UV immunosuppression. Topical application of zinc salts to mouse skin reduced UV-induced sunburn cell formation.[156] Skin from metallothionein-null mice was more sensitive to UV-induced sunburn cell formation.[157] Topical zinc was capable of inducing metallothionein in hamster skin and may explain the photoprotective effect of zinc.[158]

Plant antioxidants

Plants also have to protect themselves from the sun. In fact, they have an even greater struggle to avoid being oxidized to death because they are unable to move to avoid sunlight. Virtually all plants synthesize vitamin C[159] and vitamin E[99] to protect themselves. In addition, they synthesize flavonoids, polyphenolic compounds that are powerful AOs.[160] More than 8000 of these compounds have been identified. Many of these plant AOs are consumed in the diet and are believed to have important health-providing effects for human beings.[161] Recently, some flavonoids have been demonstrated to have potent photoprotective properties when used topically on skin, including silymarin, soy isoflavones, and tea polyphenols.

Silymarin. Silymarin is an extract of the milk thistle plant, *Silybum marianum*. Milk thistle belongs to the aster family (Asteraceae or Compositae) that includes daisies, thistles, and artichokes.[162-164] Silymarin consists of a mixture of 3 flavonoids found in the fruit, seeds, and leaves of the milk thistle plant: silybin (silibinin), silydianin, and silychristine.[162] Silybin is the main component (70%-80%) and is thought to have the most biologic activity. Ancient physicians used silymarin; since the 4th cen-

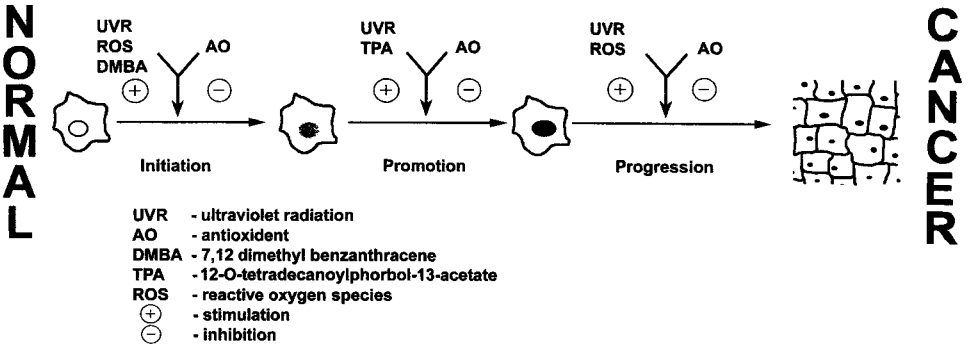


Fig 4. Multistage carcinogenesis. Skin tumors can be generated in hairless mice using series of chemicals or with ultraviolet (UV) irradiation. Each stage of process, initiation, promotion, and progression can be generated by UV irradiation, and each stage of process can be inhibited by antioxidants.

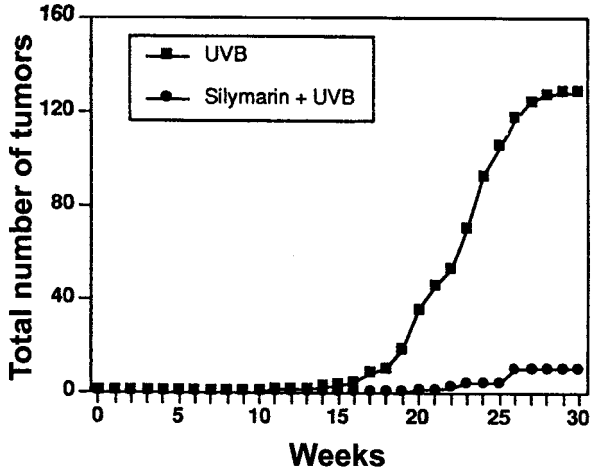


Fig 5. Silymarin protection against ultraviolet (*UV*)-induced skin tumor generation. Topical silymarin was highly effective (92% reduction) against skin tumors generated in mouse skin by UV irradiation. (Modified from Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. J Natl Cancer Inst 1997;89:556-66.)

tury BC, milk thistle extract has been used to treat disorders of the spleen, liver, and gall bladder. Silymarin has been shown to have use in many liver disorders including hepatitis, alcoholic liver disease, and cirrhosis.[165-167] It also is useful for toxin-induced liver toxicity, including poisoning from death cap mushroom (*Amanita phalloides*).[162] In an animal model of cirrhosis produced by bile duct obliteration, silymarin had an antifibrotic effect.[168] The antifibrotic effect was apparently mediated by down-regulation of procollagen a1(I), tissue inhibitor of metalloproteinase-I, and transforming growth factor β-1.[169]

Silymarin has strong AO effects. Silymarin prevented lipid peroxidation,[170-173] inhibited copper-induced low-density lipoprotein oxidation,[174] and scavenged ROS.[175-178]

Because tumor promoters cause oxidative stress (Fig 4),[179] silymarin was tested for its anticarcinogenic effects in cancer-prone SENCAR mice. It was demonstrated that low doses of topical silymarin could almost completely inhibit the effect of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter, from inducing ornithine decarboxylase activity.[180] This suggested that silymarin might have useful tumor prevention properties.

Subsequently, topical silymarin was demonstrated to have a remarkable antitumor effect (Fig 5). The number of tumors induced in the skin of hairless mice by UVB irradiation was reduced by 92% (Fig 5).[163] In addition, silymarin inhibited UVB-induced sunburn cell formation and apoptosis. Apparently the result was not related to a sunscreen effect. Topical silymarin also inhibited chemical carcinogenesis of skin tumors in SENCAR mice.[172] Tumors were initiated with 7,12 dimethyl benzanthracene (DMBA) and promoted with TPA. When tumors were initiated with DMBA and promoted with benzoyl peroxide, silymarin was also inhibitory, consistent with an AO effect as the cause of tumor inhibition.[181] Oral silymarin also effectively inhibited skin tumor growth after DMBA initiation and TPA promotion, and in addition, caused regression of established tumors.[182]

The mechanism of the anticarcinogenic effect of silymarin is unknown. Topical silymarin prevented the formation of pyrimidine dimers after UVB exposure to hairless mouse skin.[171] In human lymphocytes, silymarin protected against hydrogen peroxide–induced DNA damage as revealed by the COMET assay.[183] In cellular studies, silymarin inhibited mitogenic signaling molecules, resulting in growth inhibition and apoptosis. Thus, at low doses,

silibinin inhibited activation of the epidermal growth factor receptor and downstream mitogen-activated protein kinase-extracellular signal-regulated kinase-1 and −2 activation, resulting in growth inhibition.[184] At higher doses, apoptotic cell death occurred.[185] Silymarin inhibited cellular signal transduction. Silymarin suppressed UV-induced[186] and tumor necrosis factor-α–induced activation[187] of NF-κB without affecting AP-1. In human prostate carcinoma cells, both constitutive and tumor necrosis factor-α–induced activation of NF-κB were blocked by silibinin.[188] Inhibition was associated with an increase in inhibitory subunits of NF-κ Bα, the natural inhibitor of NF-κB, and a decrease in phospho-inhibitory subunits of NF-κ Bα; phosphorylation causes release of the inhibitor, apparently resulting from decreased I Kβ kinase activity. Silymarin has anti-inflammatory effects.[189] Inflammation was induced in skin of SENCAR mice with the tumor promotor TPA. Pretreatment with topical silymarin reduced skin edema, lipid peroxidation, and myeloperoxidase activity. Silymarin reduced TPA-induced induction of epidermal lipoxygenase, interleukin 1α, and cyclooxygenase-2 but not cyclooxygenase-1 activity. Silymarin also has antiangiogenic properties that may contribute to its anticarcinogenic effects.[190] In cultures of human vein endothelial cells, tube formation, and secretion and cell content of MMP-2/gelatinase A was inhibited by silymarin. In human prostate and breast cancer epithelial cells, vascular endothelial growth factor (VEGF) secretion was reduced by silymarin.

Soy isoflavones. Soybeans and their associated food products are a rich source of flavonoids called isoflavones. Isoflavones have attracted recent attention because epidemiologic studies have suggested that they may be responsible for the lower risk of cardiovascular disease and breast cancer in Asian populations that consume large amounts of soy.[191] In addition, these substances have estrogenic effects; phytoestrogens have been widely used in nutritional supplements to treat menopausal symptoms and postmenopausal effects, such as bone loss. For example, women in Asia have about 10% the incidence of hot flashes experienced by women in the United States.[192] Their average intake of soy is between 20 and 150 mg/d compared with 1 to 3 mg/d for women in the United States.[193]

The most plentiful isoflavones in soy are genistein and daidzein. In soy, they are present as glycosides that are converted in the gut to the free isoflavones.[194] The glycosides are not estrogenically active, which may have implications for topical use of soy.[195]

Isoflavone phytoestrogens are weak estrogens. Estrogens work by coupling with estrogen receptors (ERs) in the cell's nucleus, switching linked genes on or off. This may lead to proliferative or differentiation responses. Two types of receptors, α and β, have been identified. Both are present in skin.[196] Genistein has a 30-fold higher affinity for ERβ than ERα[197]; however, genistein in reporter studies has greater ERα agonist activity than ERβ.[198] In comparison, estradiol has 700-fold more ERα and 45-fold more ERβ activity than genistein. Even though phytoestrogens are weak estrogens, soy may contain as much as 1/1000 of its content as phytoestrogens. Circulating levels of phytoestrogens may be high, and the subsequent biologic effect may be great. Phytoestrogen receptor occupancy may potentially block the receptor and lead to antiestrogenic effects.

Skin changes dramatically during and after menopause. The thickness of the skin diminishes as does its collagen content.[199-201] Administration of oral[201,202] or topical[203,204] estrogen has been shown to increase thickness and collagen content of skin. Genistein may also have collagen-stimulating effects. In studies using skin fibroblasts, genistein increased collagen (COL1A2) gene expression.[205]

Soy isoflavones have potent anticarcinogenic effects that are largely independent of their estrogenic activities.[194] Genistein is a strong inhibitor of tyrosine kinases, which are responsible for phosphorylating proteins necessary for regulation of cell division.[206] In animal studies, oral soy or genistein protected against several cancers including bladder, breast, colon, liver, lung, prostate, and skin.[207] In cellular studies, many cancer cell lines[207] and nonneoplastic breast cells[208] were growth inhibited by genistein. Dietary soy inhibited skin tumor formation in a chemical carcinogenesis study in mice.[209] Likewise, topical genistein inhibited tumor number by 60% to 75% in mice initiated with DMBA and promoted with TPA.[210]

The nature of genistein's anticarcinogenic effect is unclear. In addition to its tyrosine kinase inhibitor effects, genistein is a potent AO. Genistein scavenged peroxyl radicals and protected against lipid peroxidation in vitro[211] and in vivo.[212] Genistein inhibited in vitro UV-induced DNA oxidation[213] and cellular DNA oxidation induced by benzopyrene and UVA,[214] psoralen plus UVA (PUVA) therapy,[215] and phorbol ester stimulation.[216] Genistein reduced hydrogen peroxide–generated DNA damage in human lymphocytes as determined by COMET assay.[217] Genistein reduced erythema and histologic inflammation induced by PUVA in mouse skin.[218] Cells containing cleaved poly (adenosine disphosphate-ribose) polymerase and active caspase-3 generated by PUVA were completely inhibited by

genistein. In addition, genistein inhibited UV-induced apoptotic changes, including caspase-3 and p21 activated kinase 2 activation in human epidermal carcinoma cells[219] and phosphokinase Cδ in human keratinocytes.[220] Genistein inhibited UVB-induced c-Fos and c-Jun expression in mouse skin, apparently by tyrosine kinase inhibition.[221] Genistein has anti-inflammatory properties. In human epidermal cell cultures, it inhibited UVB-stimulated prostaglandin E_2 synthesis[222] and suppressed UVB-induced expression of cyclooxygenase-2 in keratinocytes.[223] Finally, genistein has immune-modulating effects. Genistein inhibited UV-induced immunosuppression in mice.[224]

Tea polyphenols. Tea (*Camellia sinensis*) is a potent source of polyphenols, comprising 30% to 35% of the dry weight of the leaf. During processing, tea leaves are progressively fermented to produce green tea, oolong tea, or black tea. Green tea contains predominantly monomeric catechins including epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate. Black tea contains predominantly polymeric polyphenols.[225]

Tea polyphenols have been widely studied for their anticarcinogenic potential. They have been effective in animal models of cancer of skin, stomach, lung, esophagus, duodenum, pancreas, liver, breast, and colon.[226] However, epidemiologic studies have failed to support protection in human beings,[226] with the exception of squamous cell carcinoma of skin, where a statistically significant inverse association between skin cancer and hot black tea consumption was observed.[227]

Tea polyphenols strongly inhibit skin cancer in mouse 2-stage carcinogenesis models.[228-231] Both oral and topical green tea polyphenols decreased chemically induced[232,233] and UV-induced skin tumors.[234] Green tea also inhibited growth of established skin tumors.[235] It prevented conversion of benign skin tumors to squamous cell carcinoma.[236] Tumors were initiated by DMBA, promoted by TPA, and malignant conversion achieved by benzoyl peroxide. Green tea and black tea were equivalent in effect and decaffeinated tea was less effective.[237] Caffeine alone was effective and may importantly contribute to the effect.[238] Topical (-) epigallocatechin-3-gallate inhibited UV-induced skin tumor formation, but oral administration was ineffective.[239] Oral tea polyphenols failed to protect against basal-cell carcinoma in a UV-induced mouse model, *ptc1*$^{+/-}$.[240]

Although the nature of the anticarcinogenic effect is unknown, tea polyphenols are strong AOs,[241] more powerful than vitamin C and vitamin E.[242] They quenched singlet oxygen,[241] superoxide radical,[243] hydroxyl radical,[244-246] hydrogen peroxide,[247] and peroxyl radical.[247] They work together with vitamin E, regenerating it from its oxidation product.[248] Tea polyphenols limited UV-induced lipid peroxidation in skin[249] and reduced oxidation of proteins in a free radical–generating system in vitro.[250] Tea polyphenols regulate cellular redox-signal transduction. In human keratinocytes, (-) epigallocatechin-3-gallate inhibited UVB-induced AP-1 activity[251] and mitogen-activated protein kinase cell signaling pathways, extracellular signal-related protein kinase 1/2, c-Jun N-terminal protein kinase, and p38.[252]

Tea polyphenols are antimutagenic in microbial systems, mammalian cell systems and in vivo animal tests.[253] Tea polyphenols protected DNA from oxidation by hydrogen peroxide and UVB in vitro.[254] In human skin fibroblasts, tea polyphenols protected against radiation-induced DNA damage.[255] In Jurkat lymphocytes, epigallocatechin gallate reduced DNA damage caused by free-radical generators and hydrogen peroxide as revealed by COMET assay. [256] Topical application to skin of green tea polyphenols reduced UVB-induced pyrimidine dimers in both epidermis and dermis.[257]

Tea polyphenols induced apoptosis in several different tumor cells,[184,258] but not normal human keratinocytes that were apparently protected through induction of p57, a cell cycle regulator.[259]

Tea polyphenols may affect invasiveness of tumors. They inhibited MMPs[260-262] and inhibited adhesion of tumor cells to laminin.[263,264] Tea polyphenols may also have antiangiogenic effects. They inhibited induction of VEGF in human colon carcinoma cells[265] and inhibited VEGF-dependent VEGF receptor 2 phosphorylation in bovine aortic endothelial cells.[266]

Tea polyphenols have anti-inflammatory effects. Topical green tea polyphenols reduced UV-induced erythema and sunburn cell formation in human skin.[267] Topical (-) epigallocatechin-3-gallate reduced UVB-induced inflammatory responses and infiltration of leukocytes in human skin.[268] Green tea polyphenols also protected against erythema, and c-Fos and p53 induction after PUVA phototoxic injury to human skin.[269] Tea polyphenols also have immune-modulating effects. Green tea polyphenols protected human skin from UV-induced Langerhans cell depletion.[267] Topical (-) epigallocatechin-3-gallate protected against UVB-induced immunosuppression and tolerance in mice.[270] Topical application of (-) epigallocatechin gallate also inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice.[271]

CONCLUSION

Oxidative stress can occur from many sources in the skin including metabolism, pollution, and sunlight radiation. A wealth of information supports the photocarcinogenic damage to skin from sunlight and its relationship to oxidative stress. In animal models of photocarcinogenesis, AOs provide protection when provided to the skin systemically or topically. AOs work together in skin, supporting and regenerating each other. Topical AOs may provide several advantages for photoprotection not provided by dietary supplements. If AOs can be delivered into skin, they can be targeted to exposed skin, circumvent physiologic barriers to systemic tissue delivery, and accumulate in pharmacologic concentrations. Their presence should supplement the natural AO protection present in skin, and provide supplemental reserves as oxidative stress depletes AO stores.

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Answers to CME examination

Identification No.803-101

January 2003 issue of the Journal of the American Academy of Dermatology

Questions 1-30, Pinnell SR. J Am Acad Dermatol 2003;48:1-19.

1. e	7. d	13. a	19. e	25. e
2. c	8. e	14. e	20. a	26. e
3. e	9. c	15. e	21. b	27. d
4. e	10. d	16. e	22. d	28. a
5. b	11. e	17. d	23. a	29. e
6. a	12. c	18. e	24. d	30. b

Answer sheets are bound into the Journal for US, Canadian, and life members. Request additional answer sheets from American Academy of Dermatology, Member Services Department, PO Box 4014, Schaumburg, IL 60168-4014. Phone 847-330-0230; E-mail: tsmith@aad.org

CME examination

Identification No. 803-101

Instructions for Category I CME credit appear in the front advertising section. See last page of Contents for page number.

Questions 1-30, Pinnell SR. J Am Acad Dermatol 2003;48:1-19.

Directions for questions 1-30: Give single best response.

1. In contrast to UVB, UVA
   a. is more plentiful
   b. penetrates window glass
   c. generates more oxidative stress
   d. none of the above
   e. all of the above

2. The major chromophore in skin for the generation of singlet oxygen is
   a. DNA
   b. keratin
   c. urocanic acid
   d. selenium
   e. hemoglobin

3. C→T CC→TT DNA mutations are characteristic for
   a. oxidative damage
   b. UVA absorption
   c. p53
   d. xeroderma pigmentosum
   e. UV photodamage

4. p53
   a. monitors DNA quality control
   b. is characteristically mutated in squamous cell carcinoma
   c. is induced by UVB
   d. none of the above
   e. all of the above

5. A free radical is most likely to react with
   a. cell membranes
   b. the nearest substrate to which it is created
   c. DNA
   d. proteins
   e. lipids

6. Controlled studies have revealed that sunscreens are applied at only 20% to 25% of recommended levels. A sunscreen with a sunscreen protection factor (SPF) of 30 applied at that level would provide the following protection:
   a. SPF 2-3
   b. SPF 6-8
   c. SPF 10-15
   d. SPF 20-25
   e. SPF 30

7. In the absence of erythema, sunscreens protect skin from
   a. UV immunosuppression
   b. DNA mutation
   c. p53 induction
   d. none of the above
   e. all of the above

8. The skin uses which of the following to protect itself from the sun?
   a. L-ascorbic acid and α-tocopherol
   b. Catalase and superoxide dismutase
   c. Glutathione and ubiquinol
   d. None of the above
   e. All of the above

9. Which statement is *incorrect*?
   a. α-tocopherol protects cellular membranes
   b. L-ascorbic acid regenerates oxidized α-tocopherol
   c. α-tocopherol regenerates oxidized L-ascorbic acid
   d. Dehydroascorbic acid cannot be reduced back to L-ascorbic acid
   e. Physiologic AOs work together to protect cellular structures

10. L-ascorbic acid
    a. is synthesized in the body
    b. protects skin from sunlight by stimulating melanin synthesis
    c. stimulates elastin synthesis in skin
    d. must be un-ionized to get into skin when applied topically
    e. Is synthesized only by animals

11. Ester derivatives of vitamin C and vitamin E are often used in topical products of skin. Which statement is most true about these derivatives?
    a. They must be converted to the vitamin to be effective in skin
    b. The derivatives are not as effective as the vitamins for preventing UV photodamage
    c. Esterification often blocks the AO properties of the vitamin
    d. None of the above
    e. All of the above

12. L-ascorbic acid and α-tocopherol block UV-induced pigment formation by

a. blocking the penetration of sunlight at the skin surface
b. absorbing UV light
c. inhibiting melanin synthesis
d. oxidizing tyrosinase
e. enhancing endothelin-1

13. Selenium
a. protects the body against oxidative stress
b. protects human beings against UV-induced cell cancer
c. stimulates melanogenesis
d. None of the above
e. All of the above

14. Zinc
a. serves as a cofactor for enzymes responsible for DNA replication
b. induces metallothionein, an AO protein
c. may replace potentially damaging redox-active molecules
d. none of the above
e. all of the above

15. Plants and animals protect themselves from sunlight's photooxidative stress by synthesizing
a. vitamin C
b. vitamin E
c. polyphenols
d. none of the above
e. all of the above

16. AOs have strong antitumor effects. AOs can effect
a. tumor initiation
b. tumor promotion
c. tumor progression
d. none of the above
e. all of the above

17. Which of the following statements is *incorrect?*
a. Skin contains ERα
b. Skin contains ERβ
c. Soy phytoestrogens may block ERs
d. Soy isoflavone glycosides are estrogenically active
e. Soy isoflavone, genistein, is an inhibitor of tyrosine kinase

18. Each of the following statements is true about silymarin *except*
a. silymarin is derived from the milk thistle plant
b. oral silymarin is used as an antidote against mushroom poisoning
c. oral silymarin reduces experimental skin tumors in mice
d. topical silymarin reduces experimental skin tumors in mice
e. silymarin causes ER activation

19. Silymarin may inhibit skin tumor development by
a. preventing formation of thymine dimers
b. promoting apoptosis
c. AO effects
d. none of the above
e. all of the above

20. Topical application of each of the following reduced experimental skin tumors in mice *except*
a. zinc chloride
b. selenomethionine
c. silymarin
d. genistein
e. epigallocatechin-3-gallate

21. Each of the following are plant polyphenols *except*
a. daidzein
b. tocopherol
c. silymarin
d. genistein
e. epigallocatechin-3-gallate

22. Each of the following statements is true *except*
a. after menopause, skin becomes thinner.
b. after menopause, collagen content in skin decreases.
c. oral estrogen increases postmenopausal skin collagen.
d. oral genistein increases postmenopausal skin collagen.
e. topical estrogen increases postmenopausal skin collagen.

23. Genistein has an anticarcinogenic effect when used topically. The effect is least likely to be a result of
a. estrogenic effect
b. AO effect
c. tyrosine kinase inhibition
d. reduction of DNA damage
e. inhibition of UV immunosuppression

24. Which of the following statements is *least* correct about photocarcinogenesis?
a. UVB causes tumor initiation.
b. UVA causes tumor promotion.
c. UVB causes UV immunosuppression.
d. UVA induces cyclopyrimidine dimer formation.
e. Sunlight is a complete carcinogen.

25. Which of the following statements is *most* correct about photoaging?
a. UVA1 can cause photoaging changes.
b. DNA in mitochondria is altered by UVA.
c. Pieces of DNA can stimulate melanogenesis.
d. None are correct.
e. All are correct.

26. Which of the following statements is *most* correct about the anticarcinogenic effect of tea polyphenols?
a. Green tea inhibits skin cancer in mice.
b. Black tea inhibits skin cancer in mice.
c. Caffeine inhibits skin cancer in mice.
d. None are correct.
e. All or correct.

27. Which of the following statements about selected AOs is *least* correct?
a. α-Tocopherol is a major AO of the stratum corneum.

b. Ubiquinol protects cell mitochondria from oxidative stress.
c. L-ascorbic acid protects tissue fluids from oxidative stress.
d. α-Tocopherol protects the cytoplasm of cells from oxidative stress.
e. Vitamins C and E work together in the body to produce synergistic effects.

28. Which of the following statements is *incorrect* about UV-induced melanogenesis?
a. Oral vitamin C and vitamin E reduce the tanning response.
b. Topical vitamin C and vitamin E reduce the tanning response.
c. L-ascorbic acid inhibits tyrosinase.
d. α-Tocopherol inhibits tyrosinase.
e. Thymine dinucleotide can stimulate melanogenesis in melanocytes.

29. Sunlight produces which one of the following effects on DNA in skin?
a. Generation of cyclopyrimidine dimers
b. Oxidation of guanine to 8-hydroxyguanine
c. Inhibition of nucleotide repair
d. None of the above
e. All of the above

30. Which of the following statements is *incorrect* about UV immunosuppression?
a. Almost all patients with skin cancer have UV immunosuppression.
b. Almost all patients without skin cancer do not have UV immunosuppression.
c. Topical L-ascorbic acid blocks UV immunosuppression in mice.
d. Oral zinc reduces UV immunosuppression in mice.
e. Topical epigallocatechin-3-gallate reduces UV immunosuppression in mice.