Oxidative Stress in the Pathogenesis of Skin Disease

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Skin is the largest body organ that serves as an important environmental interface providing a protective envelope that is crucial for homeostasis. On the other hand, the skin is a major target for toxic insult by a broad spectrum of physical (i.e. UV radiation) and chemical (xenobiotic) agents that are capable of altering its structure and function. Many environmental pollutants are either themselves oxidants or catalyze the production of reactive oxygen species (ROS) directly or indirectly. ROS are believed to activate proliferative and cell survival signaling that can alter apoptotic pathways that may be involved in the pathogenesis of a number of skin disorders including photosensitivity diseases and some types of cutaneous malignancy. ROS act largely by driving several important molecular pathways that play important roles in diverse pathologic processes including ischemia–reperfusion injury, atherosclerosis, and inflammatory responses. The skin possesses an array of defense mechanisms that interact with toxicants to obviate their deleterious effect. These include non-enzymatic and enzymatic molecules that function as potent antioxidants or oxidant-degrading systems. Unfortunately, these homeostatic defenses, although highly effective, have limited capacity and can be overwhelmed thereby leading to increased ROS in the skin that can foster the development of dermatological diseases. One approach to preventing or treating these ROS-mediated disorders is based on the administration of various antioxidants in an effort to restore homeostasis. Although many antioxidants have shown substantive efficacy in cell culture systems and in animal models of oxidant injury, unequivocal confirmation of their beneficial effects in human populations has proven elusive.

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Introduction

Skin, the largest human body organ, provides a major interface between the environment and the body and is constantly exposed to an array of chemical and physical environmental pollutants (Athar, 2002). In addition, a large number of dietary contaminants and drugs can manifest their toxicity in skin (Sander *et al*., 2004). These environmental toxicants or their metabolites are inherent oxidants and/or directly or indirectly drive the production of a variety of reactive oxidants also known as reactive oxygen species (ROS). ROS are short-lived entities that are continuously generated at low levels during the course of normal aerobic metabolism. ROS include singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$) H_2O_2, the hydroxyl radical ($OH^{\cdot}$), etc. $^1O_2$ is formed by the transfer of physical or chemical energy to molecular oxygen (O_2), which at ambient temperatures behaves as a triplet and is paramagnetic. $^1O_2$ has no unpaired electrons and is a very strong oxidant. The stepwise sequential univalent reduction of O_2 leads to the formation of $O_2^{\cdot-}$, H_2O_2, and $OH^{\cdot}$. Free radical reactions differ from non-radical ones in that new radical species are generated as at least one of the reaction products. Free radical-driven reactions are usually chain reactions. For example, acting as an electron donor, $O_2^{\cdot-}$ can lead to generation of $OH^{\cdot}$ through an $O_2^{\cdot-}$-driven Fenton reaction, and by interaction with NO, can generate highly reactive peroxynitrite ($ONOO^-$). Electron acceptors such as molecular oxygen react readily with free radicals to themselves become free radicals. An additional source of oxygen radicals in skin as well as in other organs is infiltrating activated leukocytes that possess abundant systems capable of generating these species, among which are $O_2^{\cdot-}$ and hypochlorite, which are important sources of ROS *in situ.* The fundamental purpose of the release of large amounts of ROS during the inflammatory process is to kill or destroy invading microorganisms and/or to degrade damaged tissue structures. It is the imprecise targeting of ROS that can induce oxidative stress in adjacent normal cells leading to enhancement of pathologic processes (Cerutti *et al*., 1992).

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Abbreviations: AA, arachidonic acid; AP-1, activator protein 1; BCC, basal cell carcinoma; COX, cyclooxygenase; ERK, extracellular signal-regulated kinase; GSH, glutathione; GST, glutathione S-transferase; JNK, c-Jun N-terminal kinase; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloprotease; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, superoxide anion; O_2, molecular oxygen; ODC, ornithine decarboxylase; $OH^{\cdot}$, hydroxyl radical; PUVA, psoralens plus UVA; ROS, reactive oxygen species; SCC, squamous cell carcinoma; SOD, superoxide dismutase

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Fe^{2+} is an important catalyst for generating free radical-driven ROS by the Fenton reaction (Golberg *et al.*, 1962). Cu^{+} is almost as effective as iron as a catalyst in this reaction but is a more potent mutagen than iron owing to its potential to directly interact with DNA bases. Antioxidant defense systems have also co-evolved with aerobic metabolism to counteract the destructive effects of ROS to minimize their potential to cause tissue damage. In spite of these antioxidant defense mechanisms, which are likely programmed genetically, ROS-dependent damage of proteins, DNA, and other macromolecules accumulates during the lifetime of all aerobic organisms. It is known that many age-dependent diseases such as atherosclerosis, osteoarthritis, neuro-degenerative disorders, and cancer involve ROS during some stage of their progression (Serri *et al.*, 1979; Lopez-Torres *et al.*, 1994). Our laboratory has had a long-standing interest in understanding the role of cutaneous oxidative metabolism in skin carcinogenesis and assessing the feasibility of using antioxidants as anticancer agents (Bickers *et al.*, 1982; Bickers and Athar, 2000). In this brief update, we summarize current knowledge regarding the role of ROS in activating signals that are involved in the pathogenesis of inflammatory skin diseases as well as non-melanoma skin cancer.

Oxidants in skin

Skin exposure to ionizing and UV radiation and/or xenobiotics/drugs generates ROS in excessive quantities that quickly overwhelm tissue antioxidants and other oxidant-degrading pathways. Uncontrolled release of ROS is involved in the pathogenesis of a number of human skin disorders including cutaneous neoplasia (Briganti and Picardo, 2003; Black, 2004b). The agents that produce oxidative stress in skin include gaseous airborne environmental pollutants generated by automobile and other industrial sources, UV radiation, food contaminants/additives/preservatives, cosmetic products, drugs, etc. (Athar, 2002). In addition, heme pathway intermediates may have pro-oxidant effects, whereas heme oxygenase, an enzyme that degrades heme, can function as both an antioxidant and a pro-oxidant (Ryter and Tyrrell, 2000). Many of these agents may intrinsically generate ROS or their metabolites such as redox active quinones several of which may be involved in the pathogenesis of multiple skin disorders/allergic reactions/neoplasms (Briganti and Picardo, 2003; Black, 2004b; Sander *et al.*, 2004). In Figure 1, we have summarized various *in situ* biochemical reactions that generate or block oxidant production to maintain homeostatic control of intracellular redox state; imbalances can ultimately lead to oxidative stress and tissue injury. In earlier studies, we demonstrated that skin has the enzymatic machinery to convert highly hydrophobic and otherwise inert xenobiotics such as the polynuclear aromatic hydrocarbons into reactive species by oxidative metabolism catalyzed by a large family of inducible heme-containing enzymes, known as the cytochrome *P*450s (Alvares *et al.*, 1974; Bickers *et al.*, 1974; Wiebel *et al.*, 1975; Bickers and Kappas, 1978). We and others have also shown that exposure of skin to a number of chemical and physical environmental agents induces oxidative stress leading to induction of cutaneous lipid peroxidation with concomitant modulation in the levels of antioxidant and drug-metabolizing enzymes (Bickers *et al.*, 1982; Das *et al.*, 1985; Connor and Wheeler, 1987). In later studies, it was demonstrated that ROS induce a number of transcription factors such as activator protein 1 (AP-1) and NF-κB (Dhar *et al.*, 2002). Recently, Reelfs *et al.* (2004) have shown that UVA irradiation of skin fibroblast releases labile iron, which is involved in the activation of NF-κB (Reelfs *et al.*, 2004). In addition, the mitogen-activated protein kinase (MAPK) pathway is a target of oxidative stress (Kim AL *et al.*, 2005). It is interesting to note that solar UV radiation, a major cause of oxidative stress in skin, also influences these pathways in ways that closely mimic ROS. In Figure 2, the effects of ROS and solar UVA/UVB on cell signaling in skin that may be involved in the pathogenesis of various skin diseases are summarized. We have shown that UVB induces cell cycle alterations in epidermal keratinocytes similar to those evoked by ROS. In addition, parenteral administration of various antioxidants may reverse UVB-induced changes in cell cycle profile and cell cycle regulatory proteins (Bickers and Athar, 2000). Similarly, both UVB and ROS induce apoptosis in keratinocytes by altering mitochondrial membrane permeability.

Another important pathway driving cutaneous inflammation is the eicosanoids, which are generated from arachidonic acid (AA) by the enzyme prostaglandin H synthetase that generates hydroxyl-endoperoxides. The peroxidase activity of this enzyme can lead to co-oxidation of a wide range of substrates, including polycyclic aromatic hydrocarbons, which become highly reactive and can then directly interact with macromolecules including DNA (Lee *et al.*, 2003). The eicosanoids including the prostaglandins and the leukotrienes are important inflammatory mediators (see below). Another pro-oxidant enzyme present in skin is known as inducible nitric oxide synthase, which is induced in infiltrating leukocytes and other phagocytic cells, and produces NO. As mentioned above, NO interacts with ROS generated during the respiratory burst to form $ONOO^{-}$, a highly unstable reactive species that can damage DNA thereby producing point mutations, deletions, or rearrangements (Lee *et al.*, 2003; Sander *et al.*, 2004). Urocanic acid is another molecule in skin that following UVB exposure undergoes *cis–trans* isomerization and is likely involved in the immunosuppressive as well as photoaging effects of sunlight. The absorption spectrum of urocanic acid matches the action spectrum for UVB-induced immunosuppression and is associated with reduced numbers of epidermal Langerhans cells. Urocanic acid is also known to prolong skin-graft survival time, and affect natural killer cell activity (Haralampus-Grynaviski *et al.*, 2002). Assessing the enhanced production of 5α-cholesterol hydroperoxide, a marker of $^{1}O_2$ generation, reveals that UVA irradiation of *trans*-urocanic acid generates $^{1}O_2$. It is also known

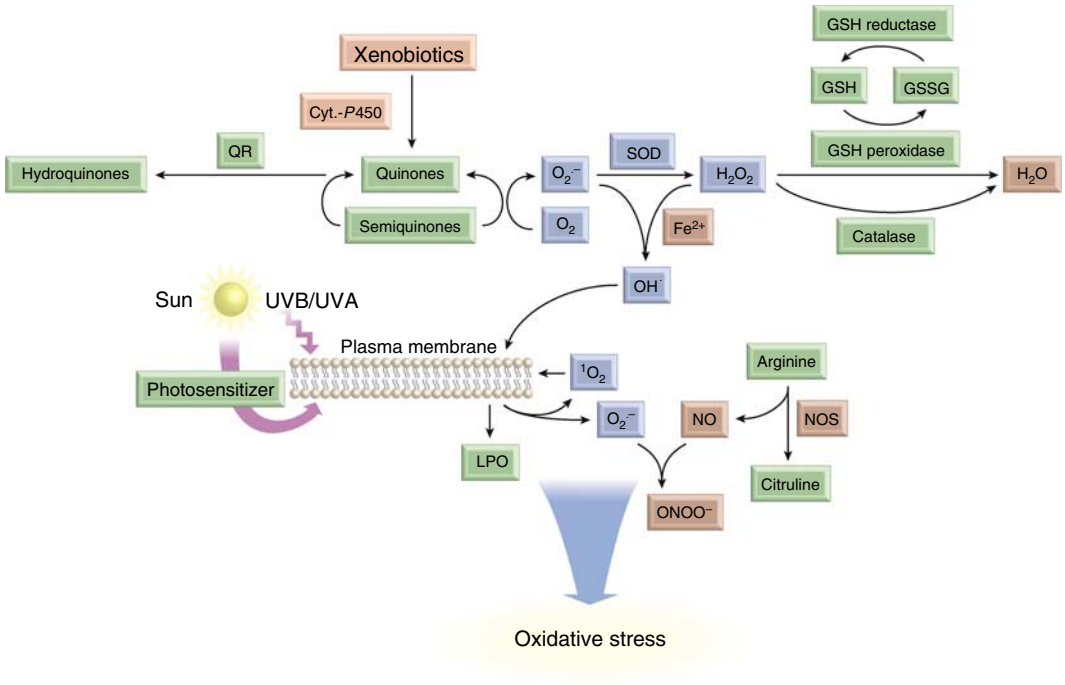

Figure 1. Generation of ROS and antioxidant defense in skin cells. Normal skin cells generate ROS such as superoxide anion ($O_2^{\cdot-}$) and H_2O_2 as a result of normal metabolism in minute concentrations. Both $O_2^{\cdot-}$ and H_2O_2 may be converted to the highly reactive hydroxyl radical ($OH^{\cdot-}$) by iron (Fe^{2+})-catalyzed Haber–Weiss and Fenton reactions. Similarly, reactive nitrogen species (RNS) are generated as a result of sequential reactions that begin with nitric oxide synthase (NOS)-mediated conversion of arginine to citrulline. In this reaction, NO is generated, which reacts with $O_2^{\cdot-}$ to produce peroxynitrite ($ONOO^-$). Similarly, ROS and RNS can be formed as a result of exposure to environmental agents including chemicals (xenobiotics) and solar UVA and UVB. Many xenobiotics are converted to toxic quinones by the family of functionally related enzymes known as cytochrome *P*450 (CYP). These quinones are redox-sensitive agents and are reversibly reduced to semihydroquinones/hydroquinones, which generate $O_2^{\cdot-}$. Both UVA and UVB produce similar free radicals and/or singlet oxygen ($^1O_2$) either directly following interaction with cellular components or in the presence of chemical agents known as photosensitizers. These photoactive chemicals while in their lowest energy or ground state absorb incident radiation (including UVA/UVB), within their absorption spectrum. The energy of the absorbed photon creates an excited state molecule, which is highly unstable under ambient conditions. In returning to the ground state, excited species transfer energy to adjacent intracellular chemical moieties particularly molecular oxygen (O_2) and thereby convert it into ROS. These ROS interact with lipid-rich plasma membranes and initiate a reaction known as lipid peroxidation. Numerous intracellular enzymes serve to degrade these reactive species. Some of these enzymes are specific such as SODs, which dismute $O_2^{\cdot-}$ to H_2O_2, whereas others have overlapping substrate affinities such as catalase and glutathione peroxidases, both of which can degrade H_2O_2 to water and O_2 but glutathione peroxidases also degrade organic peroxides to relatively non-toxic alcoholic species. These enzymes also require GSH during the course of peroxide degradation and convert GSH into its oxidized form, which is recycled by the enzyme glutathione reductase. Similarly, toxic quinones are converted to relatively less toxic hydroquinones by quinone reductases (QR).

that $^1O_2$ can initiate c-jun N-terminal kinase (JNK) signaling, which leads to interstitial collagenase induction as well as the synthesis of proinflammatory cytokines such as IL-1 and IL-6 in UVA-irradiated fibroblasts. However, this response may be modulated by endogenously generated chromophores like nicotinamide adenine dinucleotide (reduced form)/nicotinamide adenine dinucleotide phosphate (reduced form), tryptophan, riboflavin, etc. (Hanson and Simon, 1998).

Antioxidants in skin

Antioxidant molecules in the skin interact with ROS or their by-products to either eliminate them or to minimize their deleterious effects. These antioxidant molecules include glutathione (GSH), alpha-tocopherol or vitamin E, ascorbic acid or vitamin C, glutathione peroxidases, glutathione reductase, glutathione *S*-transferases (GSTs), superoxide dismutases (SODs), catalase, and quinone reductase. GSH and ascorbic acid are soluble antioxidants, whereas vitamin E is membrane-bound and capable of intercepting free radical-mediated chain reactions (Amstad *et al.*, 1991; Briganti and Picardo, 2003). GSH is present in millimolar concentrations in virtually all normal cells. However, rare mutations in human genes that encode the enzymes glutamate-cysteine ligase, glutathione synthase, and γ-glutamyl transferase in the γ-glutamyl cycle can decrease tissue and blood GSH levels (Schulman et al., 1975; Oshima *et al.*, 1976; Dalton *et al.*, 2004). In experimental animals, tissue GSH depletion can be induced by the administration of two model chemical compounds, diethyl maleate and phorone. In addition, l-buthionine-(*S*,*R*)-sulfoximine, an inhibitor of glutamate-cysteine ligase, has a similar effect (Wu *et al.*, 2004). The depletion of this tripeptide augments oxidant injury, whereas administration of agents that augment tissue GSH levels such as *N*-acetyl cysteine or 4-oxothiazoladine carboxylate affords protection against the toxic effects of ROS-generating agents (Wu *et al.*, 2004). It has also been shown that various non-phorbol ester as well as

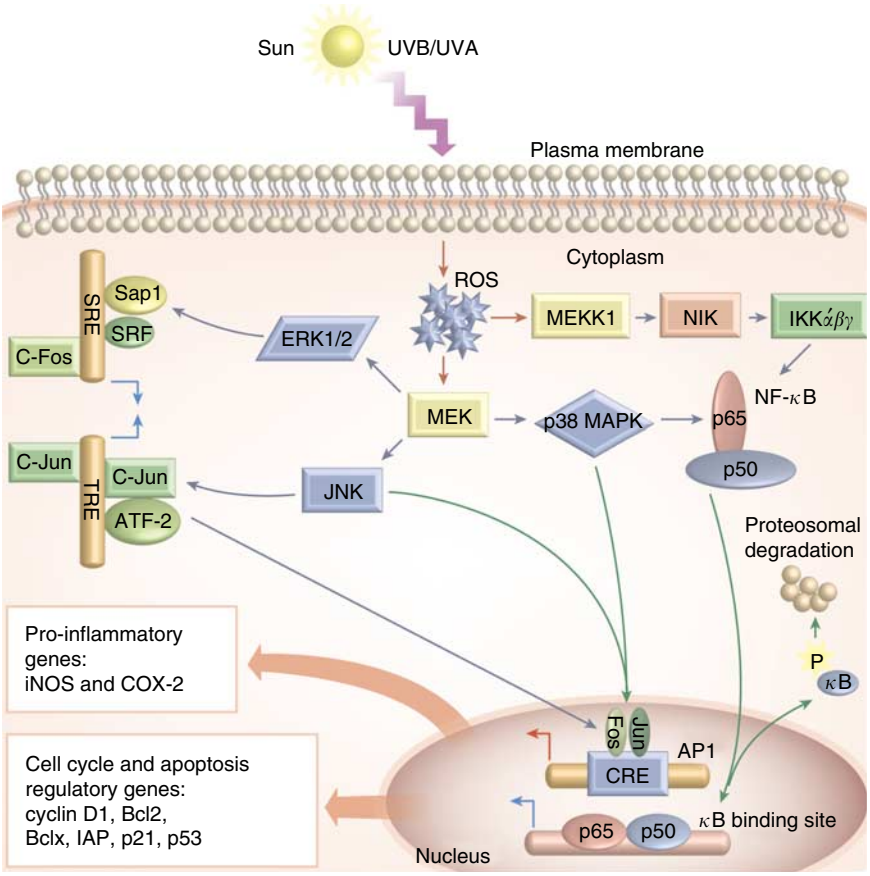

Figure 2. ROS-mediated activation of various cell signaling pathways in the skin. As a result of UVA/UVB-mediated ROS generation during the pathogenesis of various skin diseases, a number of signaling pathways are activated. ROS drive activation of MAPKs, the most important of which are ERK, JNK, and p38 kinases. ERK and JNK are important in recruiting c-Fos and c-Jun to the nucleus where they activate the transcription factor AP-1, whereas activation of p38 and inhibitory kappa kinases (IKK) is important in the transcriptional activation of NF-κB. Both of these factors are important in regulating the diverse array of genes, which play key roles in the pathogenesis of inflammation (such as iNOS, COX-2), and in regulation of cell cycle, proliferation, and apoptosis (cyclin D1, Bcl2, Bclx, IAP, p21, p53, etc.).

phorbol ester tumor promoters and UVB drive the production of ROS in murine skin (Connor and Wheeler, 1987). It has been postulated that patients with actinic keratoses and basal cell carcinoma (BCCs), the most common type of non-melanoma skin cancer, have diminished levels of antioxidant enzymes in plasma/serum (Engin, 1976). Furthermore, the SODs CuZnSOD and MnSOD are decreased in human non-melanoma skin cancers (Kobayashi *et al.*, 1991).

Role of oxidants in skin tumor development

Skin cancer is a complex multistage process that develops in three stages, initiation, promotion, and progression, which are mediated by various cellular, biochemical, and molecular changes. ROS have been shown to be involved in all three stages. Initiation is the first stage of carcinogenesis and involves the induction of structural alterations in DNA that create mutations. Genetic alterations in proto-oncogenes and tumor suppressor genes may render epidermal cells resistant to signals for terminal differentiation. At this stage, ROS may induce extensive DNA damage, which includes DNA base damage, DNA single-strand and double-strand breaks, crosslinking between DNA and proteins, or DNA and chromosomal aberrations that may be mutagenic (Athar, 2002). ROS may also be involved in tumor initiation by activating procarcinogens to generate free radicals that can attack nucleophiles (Cerutti *et al.*, 1992). One of the major products of oxidative base damage in DNA is thymine glycol that results from either chemical oxidation or ionizing radiation. It has been shown that chemical carcinogens capable of generating free radicals often induce the formation of thymine glycol (Nishigori *et al.*, 2004). Oxidative stress induces 8-hydroxyguanosine formation both in genomic and mitochondrial DNA (Athar, 2002). Elevated levels of 8-hydroxyguanosine in blood and urine of experimental animals and in humans are thought to be reliable markers of oxidative damage. Similarly, free radicals in cigarette smoke condensate are skin carcinogens in mice (Curtin *et al.*, 2004). Peroxyl radicals, which are formed by spontaneous or enzyme-catalyzed oxidation of unsaturated fatty acids may activate carcinogens such as benzo(a)pyrene, aromatic amines (e.g. naphthylamine, acetylaminofluorene, etc.), amino azo compounds, 4-nitroquinoline-1-oxide, and *n*-nitro compounds (Athar, 2002). Azathioprine, a widely used immunosuppressant drug, generates oxygen radicals when exposed to UVA. Azathioprine causes the accumulation of 6-thioguanine in DNA by forming guanine sulfonate which is a replication-blocking DNA 6-thioguanine photoproduct, and could be bypassed by error-prone, Y-family DNA polymerases *in vitro*. Biologically relevant doses of UVA generate ROS in cultured cells with 6-thioguanine-substituted DNA and 6-thioguanine and UVA are synergistically mutagenic. Drugs causing chronic oxidative stress may therefore carry a risk of therapy-related cancer and may contribute to the prevalence of skin cancer in azathioprine-treated patients (O'Donovan *et al.*, 2005). Other therapeutic modalities have been found to accelerate the growth of human skin cancer. For example, in a 20-year prospective study, multiple squamous cell carcinomas (SCCs) developed in UVA-exposed sites of patients with psoriasis receiving treatment with orally administered 8-methoxypsoralen followed by UVA (PUVA) (Stern and Lunder, 1998; Parrish, 2005).

The second stage of carcinogenesis is tumor promotion that involves clonal expansion of initiated cells. The role of free radicals in tumor promotion has been suggested based on the evidence that a number of free radical-generating compounds are tumor promoters; many tumor promoters are known to induce ROS and ROS can mimic the biochemical effects of known tumor promoters. In addition, tumor promoters can modulate tissue levels of antioxidants and/or ROS scavengers/detoxifiers and antioxidants can inhibit tumor promotion (Nakamura *et al.*, 1985) as depicted in Figure 1.

The third stage of carcinogenesis is known as tumor progression during which benign papillomas are converted into malignant neoplasms. We and others have shown that the low frequency of spontaneous conversion of papillomas to carcinomas can be increased by treating papilloma-bearing mice with free radical-generating compounds (Athar *et al.*, 1989b; Athar, 2002). In a two-stage carcinogenesis model, exposure to ionizing radiation as a source of free radicals augmented malignant conversion. We showed that organic hydroperoxides are metabolized into free radicals by SCC13 epidermal carcinoma cells (Athar *et al.*, 1989c). These observations suggest that pro-oxidant compounds are capable of metabolic conversion into free radicals that enhance malignancy. The role of oxidative stress in tumor progression is also supported by the fact that diethylmaleate, a GSH depleter, enhances tumorigenesis, whereas GSH itself or disulfiram, a compound that augments GSH levels, decreases the rate of skin tumor progression (Rotstein and Slaga, 1988). Furthermore, the free radical scavenger, *N*-acyl dehydroalanin, inhibits carcinogenesis, although it has no effect on 12-*O*-tetradecanoylphorbol-13-acetate-mediated ornithine decarboxylase (ODC) induction or papilloma formation (Sander *et al.*, 2004).

Early responses following skin exposure to UVB include induction of oxidative stress and of the enzyme ODC that drives the production of polyamines that are potent enhancers of cell proliferation. Pretreatment of mice with antioxidants abrogates both oxidative stress and ODC induction, suggesting that induction of the enzyme is downstream of oxidative stress (Sander *et al.*, 2004). Various oxidants and free radical-generating chemicals induce ODC activity in murine skin and are potent tumor promoters and augment conversion of benign papillomas to SCCs (Athar, 2002). In addition, exposure to radiant energy induces free radicals and augments oxidative stress in murine and human skin (Nishigori *et al.*, 2004). It is known that free radical scavengers and antioxidants have chemopreventive effects against chemically, physically, or biologically induced cancers in murine models of carcinogenesis (Athar *et al.*, 1989b; Athar, 2002; Sander *et al.*, 2004). Vitamin E, a potent inhibitor of lipid peroxidation, and polyphenolic antioxidants such as epicatechins derived from green tea and resveratrol extracted from the skin of grapes and other fruits afford protection against UVB-induced skin damage, ODC induction, and tumorigenesis in murine models of SCC development (Liebler and Burr, 2000). It has been demonstrated that ODC induction is regulated by protein kinase C. Both oxidant and non-oxidant tumor promoters and UVB induce protein kinase C although different mechanisms are involved (Athar, 2002; Briganti and Picardo, 2003; Black, 2004b).

NF-κB is a ubiquitous transcription factor involved in proliferative signaling and tumor promotion and is activated by oxidants and other stimuli known to generate ROS (Dhar *et al.*, 2002) as shown in Figure 2. In its resting state, NF-κB exists in the cytoplasm of the majority of cells as homo- or heterodimers of a family of structurally related proteins known as Rel or Rel/NF-κB. Cytoplasmic sequestration of NF-κB is regulated by its binding to an inhibitory protein known as I*k*B. Signals that induce transcriptional activation of NF-κB dissociate I*k*B allowing Rel/NF-κB to translocate to the nucleus (May and Ghosh, 1997). The promoter region of the ODC gene has NF-κB response elements. Thus, ROS- and UVB-mediated ODC induction may also be driven by the activation of this transcription factor (Janssen *et al.*, 1993).

To further define the role of oxidative stress in skin carcinogenesis, a number of genetically engineered mice overexpressing antioxidant enzymes or mice with these enzymes knocked out have been developed (Lu *et al.*, 1997; Long *et al.*, 2001; Dalton *et al.*, 2004; Iskander *et al.*, 2004, 2005; Elchuri *et al.*, 2005; St Clair *et al.*, 2005). The carcinogenesis experiments with these mice have yielded mixed results. The tumor yield or incidence in two-stage skin chemical carcinogenesis in mice overexpressing CuZnSOD or glutathione peroxidase or both was found to be no different from that observed in their wild-type littermates (Lu *et al.*, 1997; Elchuri *et al.*, 2005). However, overexpression of MnSOD reduced the onset and multiplicity of skin tumors (St Clair *et al.*, 2005), although in MnSOD knockout mice, tumorigenesis was not enhanced (St Clair *et al.*, 2005). Deficiency of quinone reductase 1 and 2 also enhanced tumor development in a two-stage skin chemical carcinogenesis protocol (Long *et al.*, 2001; Iskander *et al.*, 2004, 2005).

Role of oxidants in skin diseases

There is compelling evidence that oxidative stress drives the production of oxidation products, such as 4-hydroxy-2-nonenal or malonaldehyde (Meffert *et al.*, 1976), which can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators, such as cytokines, which may be critical for the induction of some inflammatory skin diseases (Meffert *et al.*, 1976). This is also based on the recognition that ROS can act as second messengers in the induction of several biological responses, such as the activation of NF-κB or AP-1, the generation of cytokines, the modulation of signalling pathways, etc. (Briganti and Picardo, 2003). The recent demonstration that the peroxisome proliferator-activated receptors, whose natural ligands are polyunsaturated fatty acids and their oxidation products may be involved in the pathogenesis of psoriasis or acne, has further strengthened the concept that ROS can drive the development of these disorders (Okayama,

2005). Vitiligo is a depigmenting disorder in which epidermal melanocytes are destroyed by as yet undefined mechanisms. It has been proposed that ROS are generated in melanocytes exposed to phenolic/catecholic derivatives and that melanocytes in patients with this disease are more susceptible to oxidant stress (Boissy and Manga, 2004).

ROS may also participate in the pathogenesis of allergic reactions in the skin. During their first encounter with antigen, memory T-lymphocytes differentiate into cytokine-producing effector cells. Two types of effector cells characterized by their distinct cytokine expression profiles are Th1 and Th2 cells. Th1-lymphocytes secrete IL-2 and IFN-γ, whereas Th2-lymphocytes produce IL-4, IL-5, IL-6, IL-10, and IL-13. Recently, it was shown that Th1 and Th2 response patterns in antigen-presenting cells are modulated by GSH (Kidd, 2003). Positive patch tests in patients who are skin contact allergic to nickel manifest increased tissue iron and an elevated oxidized/reduced GSH ratio both of which characterize oxidative stress in skin (Kaur *et al.*, 2001). Nickel enhances tissue iron and $OH^{\cdot}$ generation (Athar *et al.*, 1987). In a separate study, it was shown that induction of allergic contact dermatitis to polyaromatic hydrocarbons requires metabolic activation by cytochrome *P*450-dependent enzymes (Anderson *et al.*, 1995). Similarly, contact allergic responses to paraphenylenediamine may be regulated by cytochrome *P*450-dependent generation of an oxidation product of paraphenylenediamine known as a Bandrowski base (Kawakubo *et al.*, 1997). There is evidence that genes within the major histocompatibility complex influence cell-mediated immunity to some carcinogenic chemicals and may serve to protect individuals by removing mutant cells before they can develop into neoplasms (Elmets *et al.*, 1998). It was recently reported that ROS upregulate dendritic cell surface markers, including major histocompatibility complex class II molecules, suggesting that antigen-specific, bidirectional dendritic cell–T-cell communication can be blocked by interfering with redox regulation pathways. ROS may play an important homeostatic role in activation of sentinel dendritic cells, linking tissue damage to the initiation of an immune response (Briganti and Picardo, 2003). Addition of DNFB, a strong skin sensitizer, to a dendritic cell line generated from fetal mouse skin enhanced protein oxidation and induced p38 MAPK and extracellular signal-regulated kinase (ERK)1/2 phosphorylation, which could be blocked by GSH (Matos *et al.*, 2005).

ROS trigger induction and maintenance of cutaneous inflammation (Trenam *et al.*, 1992). Skin exposure to a number of irritants or proinflammatory agents including UVA and UVB generates ROS through the oxidative burst in infiltrating leukocytes at the site of inflammation (Black, 2004a). Exposure of keratinocytes to chemical irritants, allergens, or inflammatory stimuli triggers activation of several stress-sensitive protein kinases, involving ROS as mediators, leading to enhanced elaboration of cytokines. ROS directly alter kinases, phosphatases, and transcription factors, or modulate cysteine-rich redox-sensitive proteins. In human keratinocytes, ROS enhance EGFR phosphorylation and activate ERKs and JNKs (Maziere *et al.*, 2003). Families of MAPKs include p38, as well as ERK, and JNK all of which exhibit extensive crosstalk among themselves. However, the ERK pathway primarily mediates cellular responses to growth factors, whereas the JNK and p38 pathways primarily mediate cellular responses to cytokines and physical stress. It has been suggested that the dynamic balance between the growth factor-activated ERKs and the stress-activated JNKs and p38 pathways may be important determinants of cell survival in the face of stress. Downstream effectors of the MAPKs include several transcription factors such as Elk-1, Ets, CREB, c-Fos and c-Jun. c-Jun and c-Fos heterodimerize to form the AP-1 complex. Phosphorylation of c-Jun by JNK stimulates AP-1 transactivation activity (Shin *et al.*, 2005). These workers also showed that aging skin fibroblasts have decreased catalase activity. Accumulation of ROS owing to catalase attenuation may be a critical aspect of the MAPK signaling changes that result in skin aging and photoaging in human skin *in vivo* (Fisher *et al.*, 2002). UVB activates ERK1/2 and p38 signaling in epidermal keratinocytes via ROS generation (Kim AL *et al.*, 2005). p38 MAPK signaling pathway is activated by UVB in murine skin (Kim AL *et al.*, 2005). UVB-induced phosphorylation of p38 MAPK enhances both the level and activity of MAPK-activated protein kinase-2. MAPK-activated protein kinase-2 activation results in the phosphorylation of its substrate, heat-shock protein 27. Oral administration of the p38 inhibitor SB242235 to mice, before UVB irradiation, blocks activation of the p38 MAPK cascade, and abolishes MAPK-activated protein kinase-2 kinase activity and phosphorylation of heat-shock protein 27 in addition to inhibiting the expression of the proinflammatory cytokines IL-6 and KC (murine IL-8) and cyclooxygenase (COX)-2 (Kim AL *et al.*, 2005). Aging skin shows downregulation of ERKs, whereas stress-activated MAPKs are augmented. Similar to other inflammatory responses, UVA-induced skin inflammation shows ROS-dependent activation of NF-κB through the degradation of its regulatory IκBα protein. However, UVA also releases free iron from *in situ* iron stores, which also acts as an IκBα-independent activator of NF-κB (Bachelor and Bowden, 2004). UVA-induced NF-κB is involved in the transcriptional regulation of a number of proinflammatory signals in skin (Hsu *et al.*, 2000). UVA also variably activates ERKs, JNKs, and p38 kinases. In human keratinocytes, UVA phosphorylates and activates all three MAPKs, whereas in human skin fibroblasts, UVA had similar effects on JNKs and p38 kinases but not ERKs (Bode and Dong, 2003). Furthermore, UVA activation of p38 and JNKs diminishes in the presence of scavengers of singlet oxygen. Finally, it is known that UVA can activate and phosphorylate ribosomal S6 kinases and this may occur either through EGFR or by signaling through phosphatidylinositol-3 kinase (Bode and Dong, 2003).

Varicose ulcers may be another example of oxidant-stress driven

pathology. These ulcers are characterized by chronic inflammation in which heme and iron are deposited in the tissue. Histochemical analysis of chronic wound tissue also shows the presence of iron deposits, heme/porphyrins, in infiltrating cells, basement membranes, and fibrin cuffs around blood vessels (Allhorn *et al.*, 2003).

Drug-induced skin photosensitization is another category of inflammatory response that involves generation of ROS (Briganti and Picardo, 2003). Photodynamic therapy combines the use of porphyrins as photosensitizers and exposure to light to generate ROS that can damage/destroy tumor cells (Athar *et al.*, 1988). However, one major drawback of photodynamic therapy is the prolonged half-life of many of the available photosensitizers in the skin that can then cause protracted cutaneous photosensitivity. We showed that $O_2^{\cdot-}$ and other ROS are involved in this process (Athar *et al.*, 1988). Thus, cutaneous porphyrin photosensitization requires the generation of $O_2^{\cdot-}$ and various other ROS as ascertained by electron spin resonance spectroscopy. In this process, $O_2^{\cdot-}$ can be generated by the enzyme xanthine oxidase. Evidence supporting this concept comes from studies showing that allopurinol, a potent inhibitor of xanthine oxidase, blocks porphyrin-mediated photosensitivity responses (Athar *et al.*, 1989a).

Dietary intervention and metabolic disposition

Preventive strategies to reduce the damaging effects of ROS in driving carcinogenesis are being extensively studied worldwide. Tea extracts have greater antioxidant activity than do most vegetables and fruits and may be more potent antioxidants than vitamin C or E or carotenoids. More importantly, tea is a ubiquitous non-toxic substance with human consumption dating back several millennia. Over the past two decades, our laboratory has been exploring the feasibility of exploiting the potent antioxidant properties of tea and its constituents as skin cancer chemopreventive agents. Extracts of tea contain numerous polyphenols that act as antioxidants to modulate carcinogen metabolism, trap reactive electrophilic metabolites, scavenge free radicals, inhibit cell proliferation, arrest the cell cycle, and induce apoptosis. In our early studies, it was shown that green tea treatment inhibits cytochrome *P*450-dependent mono-oxygenase activity in skin microsomes. It also induces phase II detoxification enzymes through its interaction with an antioxidant responsive element located at the 5′ flanking region of phase II drug-metabolizing genes that mediate glucuronidation and sulfation. Green tea inhibits polyaromatic hydrocarbon-induced skin tumor initiation, decreasing 7,8-dihydroxy-9,10 epoxy-7,8,9,10 tetrahydrobenzo(a)pyrene-induced skin tumorigenesis and UVB-induced photocarcinogenesis in multiple murine models. Both black and green tea and their constituents inhibit all three stages of skin carcinogenesis including initiation, promotion, and malignant progression. In addition, oral administration of green tea in drinking water enhances regression and retards growth of 7,12-dimethylbenz[a]anthracene- or UVB-initiated and 12-*O*-tetradecanoylphorbol-13-acetate-promoted skin papillomas (Bickers and Athar, 2000).

The combination of photosensitizing drugs known as psoralens and UVA (PUVA) has been used extensively for treating patients with psoriasis and cutaneous T-cell lymphoma. PUVA causes structural damage to DNA and can generate ROS such as $O_2^{\cdot-}$ that are clastogenic (Filipe *et al.*, 1997). This may contribute to the increased risk for developing SCCs and melanoma in the skin of PUVA-treated patients. Studies have shown that orally administered green tea extract before or during multiple PUVA treatments of SKH-1 hairless mice reduces hyperplasia, hyperkeratosis, erythema, and edema. In addition, surrogate biomarkers of skin injury and proliferation such as c-fos, p53, and proliferating cell nuclear antigen induced by PUVA are abrogated by green tea administration (Zhao *et al.*, 1999). Several experimental studies conducted in human skin have verified the efficacy of tea constituents as inhibitors of carcinogenesis-associated surrogate markers of inflammation. Topical application of black tea extracts reduces UVB-induced erythema and edema in human skin (Bickers and Athar, 2000). UVB-induced proinflammatory signaling is abrogated by the administration of green tea polyphenols. Similarly, epigallocatechin-3-gallate binds with EGFR and suppresses extracellular signaling leading to inhibition of cell proliferation and induces G1 arrest leading to apoptosis (Bowden, 2004). Black tea extracts inhibit UVB-induced tyrosine phosphorylation of EGFR and inhibit UVB-induced expression of early response proto-oncogenes such as c-fos, c-jun, p53, etc. In lipopolysaccharide-induced inflammation, epigallocatechin-3-gallate blocks NF-κB and dependent nitric oxide synthase activity (Bowden, 2004).

Resveratrol is another potent naturally derived antioxidant that has been studied for its cancer chemopreventive effects in skin. Resveratrol exerts its anticarcinogenic effects by causing cell cycle arrest and inducing apoptosis in various types of malignant cells. The induction of apoptosis is driven by effects on the tumor suppressor p53. For example, treatment of several thyroid cancer cell lines with resveratrol caused activation and nuclear translocation of ERKs that was associated with increased p53 phosphorylation (Ser15) and accumulation of p53 protein and apoptosis (Bode and Dong, 2004). Resveratrol was also shown to induce apoptosis and growth arrest in HCT-116 cells (Bode and Dong, 2004). These effects corresponded with enhanced expression of antitumorigenic NAG-1 (non-steroidal anti-inflammatory (NSAID) drug-activated gene-1), a member of the transforming growth factor-β superfamily mediated by p53. Structural analogs of resveratrol specifically inhibit the growth of transformed WI38 cells, but had little effect on normal WI38 cells (Bode and Dong, 2004). The growth inhibition was linked to increased expression of p53, GADD45, and Bax with corresponding suppression of Bcl2. Dong and co-workers found that resveratrol-induced activation of p53 and apoptosis depends on the activities of ERKs and p38 kinase and their

phosphorylation of p53 at serine 15 (Bode and Dong, 2004). In addition, resveratrol activates JNKs. Stable expression of a dominant-negative mutant of JNK1 or disruption of the *Jnk1* or *Jnk2* gene markedly inhibited resveratrol-induced p53-dependent transcriptional activation and induction of apoptosis (Bode and Dong, 2004).

The GSTs are a supergene family of dimeric enzymes that catalyze the conjugation of GSH to a variety of electrophiles including arene oxides, unsaturated carbonyls, organic halides, and other substrates (e.g. by-products of ROS activity). These enzymes are ubiquitously present in living organisms. A number of chemical agents including some antioxidants and tea compounds that have cancer chemopreventive properties can induce one or more GST isozymes (Lear *et al.*, 1997). Interestingly, recent data have suggested a role, at least for the pi class gene product, in JNK inhibition. In addition, these enzymes are also associated with various polymorphic allelic variants that in humans have been associated with susceptibility to various diseases (Ramachandran *et al.*, 2001). It has also been shown that these genotypes modify disease phenotype (Ramachandran *et al.*, 2001). The influence of GST polymorphisms has been associated with augmented risk of several cancers, including BCCs. For example, both GSTM1 and GSTT1 genotypes are associated with increased susceptibility to BCCs and GSTT1 null was found in a subgroup of BCC patients who developed large numbers of primary tumors in clusters (Ramachandran *et al.*, 2001).

The multiple targets of action of tea and its constituent polyphenols and of resveratrol provide a strong rationale to pursue studies to verify the usefulness of these agents in diminishing the risk of human skin cancer. Additional agents with potential antioxidant properties that have shown efficacy in murine models of skin carcinogenesis include curcumin, silymarin, genistein, apigenin, ascorbic acid, and garlic derivatives. These agents have shown variable efficacy as inhibitors of inflammation, as well as tumorigenesis induced by chemicals or UV radiation (F'Guyer *et al.*, 2003). They may act on multiple targets and block multiple pathways related to cell cycle regulation, MAPKs, transcription factors such as AP-1 and NF-κB and activate p53-dependent and p53-independent proapoptotic pathways.

Augmented oxidative stress is known to activate phospholipase A2, which releases membrane-bound AA. The oxidative metabolism of AA is catalyzed by among others two major enzymes known as COX and lipoxygenases (LOX), which produce prooxidant metabolites classified as eicosanoids (respectively as prostaglandins and hydroxyeicosatetraenoic acids). Many of these AA metabolites are clastogenic and act as tumor promoters in murine models of skin carcinogenesis and are induced following UVB irradiation. COX is the key enzyme generating prostaglandins from AA. In humans, prostaglandins are involved in diverse physiological functions. At least two isoforms of COX have been cloned and sequenced. COX-1 is a housekeeping isoform constitutively expressed in most tissues, whereas COX-2 is induced by a variety of proinflammatory agents and mitogens. It is known that COX-2 is upregulated following acute UVB exposure, and is increased in human actinic keratoses/papillomas and in both murine and human SCCs. In contrast, BCCs show little or no COX-2 expression in tumor islands, whereas there is irregular expression in tumor stroma. In addition, topical administration of the COX-2 inhibitor celecoxib is effective in attenuating acute UVB-induced inflammation and associated COX-2 expression. Green tea extracts have similar effects (Lee *et al.*, 2003). Carcinogenesis studies in COX-2 transgenic and knockout murine models have further verified its critical role in the pathogenesis of skin cancer. Mice deficient in COX-1 or COX-2 show premature keratinocyte terminal differentiation and develop 75% fewer tumors compared to their wild-type littermates when subjected to a 7,12-dimethylbenz[a]anthracene/12-*O*-tetradecanoylphorbol-13-acetate two-stage chemical carcinogenesis protocol (Lee *et al.*, 2003). Furthermore, transgenic mice with keratin-5 promoter-driven COX-2 overexpression in basal epidermal cells exhibit a preneoplastic skin phenotype including epidermal hyperplasia, dysplasia, and increased vascularization, which disappears when these mice are fed the COX-2 inhibitor valdecoxib (Marks *et al.*, 2003). Spontaneous skin tumor development rarely occurs in these mice, suggesting that COX-2 overexpression alone is not sufficient to induce tumorigenesis. However, when these mice were treated with the tumor initiator 7,12-dimethylbenz[a]anthracene, numerous tumors develop even without 12-*O*-tetradecanoylphorbol-13-acetate treatment, which is essential for tumorigenesis in wild-type animals. Paradoxically, keratin 14 promoter-driven COX-2 overexpression in skin led to suppression of chemically induced tumor development (Marks *et al.*, 2003). The observed differences in the K-14-COX-2 and K-5-COX-2 transgenic mice in terms of their tumor susceptibility suggest that subtle differences in COX-2 expression in the epidermis may have a decisive influence on the risk of skin carcinogenesis. Sulindac, a COX inhibitor, reduces UVB-induced skin inflammatory responses and also UVB-induced surrogate markers of skin carcinogenesis (Lee *et al.*, 2003).

LOX are a family of non-heme iron dioxygenases that regio- and stereospecifically insert molecular oxygen into polyunsaturated fatty acid substrates generating 5*S*-, 8*S*-, 12*S*-, 12*R*-, or 15*S*-hydroperoxyeicosatetraenoic acids and, on reduction, the corresponding hydroxy derivatives (HETE) with AA and 9*S*- or 13*S*-hydroperoxyoctadecadienoic acids and the corresponding hydroxy derivatives (HODE) with linoleic acid as a substrate (Steele *et al.*, 1999). There are several isozymes of LOX. The isozymes *e*12*S*-LOX and 12*R*-LOX, the mouse 8*S*-LOX and its human orthologue 15*S*-LOX-2, and *e*LOX-3 are preferentially expressed in murine and human epidermis (Muller *et al.*, 2002). The *e*12*S*-LOX can be detected in all epidermal cell layers, whereas other LOX isoforms are only detectable in suprabasal layers of murine epidermis. Overexpression of the

LOX isoforms 8*S*- and *p*12*S*-LOX occurs in papillomas and SCCs, leading to accumulation of the corresponding metabolites 8*S*- and 12*S*-HETE. Both LOX products are known to induce chromosomal damage in primary basal murine keratinocytes (Muller *et al.*, 2002). However, the amounts of these metabolites formed in tumors are sufficient for the formation of etheno adducts of DNA. Therefore, 8*S*- and 12*S*-HETE may generate endogenous mutagens. Nordihydroguaretic acid, a potent common inhibitor of these enzymes, suppresses skin tumor induction in murine models (Muller *et al.*, 2002).

Recently, it has been shown that in transgenic mouse lines that differentially express *e*12*S*-LOX, low transgene expression correlates with a decreased skin tumor response whereas high transgene expression coincides with increased tumor development (Kim E *et al.*, 2005). However, gain-of-function studies with 8*S*-LOX/8-HETE show that increased 8-HETE production in normal and tumorigenic keratinocytes drives differentiation and cell cycle arrest leading to reduced tumor yield (Kim E *et al.*, 2005).

Matrix metalloproteases (MMPs) are another category of enzymes important in the pathogenesis of skin diseases and aging. These proteases degrade macromolecules of the extracellular matrix including collagen and elastin. Currently, at least 20 MMPs are known (Brenneisen *et al.*, 2002). Their activity in normal tissue is low, but during the progression of several pathological states including cancer and aging, activity increases leading to malfunction of normal connective tissue remodeling (Brenneisen *et al.*, 2002). The catalytic activity of MMPs is known to be affected by ROS and also by reactive nitrogen species in some cases (Nelson and Melendez, 2004). The interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) are two major members of this large family, which appear to be important in skin carcinogenesis and aging including photoaging. Both of these MMPs are induced by UVB. The promoter region of both MMP-1 and MMP-3 are similar and carry AP-1 sites (Polte and Tyrrell, 2004). Thus, these genes are transactivated by binding of active AP-1. Redox regulation activates an MAPK signaling pathway that modulates the expression of a number of transcription factors including AP-1 and NF-κB, both of which play an important role in the activation of MMPs. A number of antioxidants such as *N*-aceyl cysteine, resveratrol, and tea polyphenols inhibit ROS-regulated expression of MMPs (Polte and Tyrrell, 2004).

Summary and perspectives

Ironically, oxygen is essential for life as we know it and is ultimately responsible for aging and death. ROS are ubiquitous in nature and are constantly produced in low amounts in aerobic systems. Living cells possess a range of antioxidant pathways that efficiently eliminate/inactivate these species to maintain homeostasis. The body is exposed to numerous pro-oxidants in the environment including among others drugs, solar radiation, pollutant chemicals, food additives, cosmetic products, etc. capable of generating ROS in skin. These species can target lipid-rich membranes as well as cellular DNA and proteins to produce an array of toxic effects. Peroxidation of lipid-rich membranes alters their fluidity and their signaling efficiency leading to inflammatory changes and to aberrant cell proliferation responses. Similarly, oxidant-mediated alterations in cellular proteins can augment death signaling pathways. These alterations may contribute to numerous skin disorders ranging from photosensitivity to cancer. This has led to a search for nontoxic antioxidants that could potentially contain and/or reverse these changes (Black, 2004b; F'Guyer *et al.*, 2003). Despite a large body of knowledge in cell culture systems and in animal models demonstrating the protective effects of a spectrum of antioxidants, it is unfortunate that no satisfactory agent has so far been developed with unequivocal efficacy in humans. Explanations for this could include the fact that (1) ROS affect different pathways in different situations and an antioxidant focused on one such pathway may be ineffective in a redundant pathway, (2) ROS pharmacokinetics in the target tissue may not relate to that of the antioxidant, and (3) bioavailability and target organ concentration of the antioxidant may be a limiting issue. Future research should be able to address these issues.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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