ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 4, 2002

Review

Oxidative Stress and Its Role in Skin Disease

KEVIN J. TROUBA,[1] HISHAM K. HAMADEH,[2] RUPESH P. AMIN,[3] and DORI R. GERMOLEC[1]

ABSTRACT

Skin is a major target of oxidative stress due to reactive oxygen species (ROS) that originate in the environment and in the skin itself. ROS are generated during normal metabolism, are an integral part of normal cellular function, and are usually of little harm because of intracellular mechanisms that reduce their damaging effects. Antioxidants attenuate the damaging effects of ROS and can impair and/or reverse many of the events that contribute to epidermal toxicity and disease. However, increased or prolonged free radical action can overwhelm ROS defense mechanisms, contributing to the development of cutaneous diseases and disorders. Although ROS play a role in diseases such as skin cancer, their biological targets and pathogenic mode of action are still not fully understood. In addition, strategies useful in the therapeutic management of ROS action in human skin are still lacking. This review is intended to give investigators an introduction to ROS, antioxidants, two skin disorders influenced by ROS action (skin cancer and psoriasis), and relevant model systems used to study ROS action. *Antioxid. Redox Signal.* 4, 665–673.

INTRODUCTION

THE SKIN is a biological barrier that defends against multiple environmental insults. Free radicals, one form of insult, induce or contribute to adverse effects on the skin, including erythema, edema, wrinkling, photoaging, inflammation, autoimmune reactions, hypersensitivity, keratinization abnormalities, preneoplastic lesions, and skin cancer (56). Pollution, atmospheric gases, ultraviolet (UV) radiation, microorganisms, viruses, and xenobiotics all serve as sources of exogenously or environmentally derived free radicals (22), whereas endogenous radicals are generated during normal cellular metabolism, immune reactions, and under pathological conditions (62). Reactive oxygen species (ROS) are a family of oxygen-based free radicals that contain or are capable of producing an unpaired electron. ROS are produced during the reduction of molecular oxygen in the intermediate steps:

$$O_2 + 1\,e^- + H^+ \rightarrow H_2O \rightarrow H^+ + \cdot\mathbf{O_2}^- \quad (1)$$

$$H_2O + 1\,e^- + H^+ \rightarrow \mathbf{H_2O_2} \quad (2)$$

$$\mathbf{H_2O_2} + 1\,e^- + H^+ \rightarrow H_3O_2 \rightarrow H_2O + \mathbf{OH}\cdot \quad (3)$$

$$\mathbf{OH}\cdot + \mathbf{1\,e^-} + \mathbf{H+} \rightarrow H_2O \quad (4)$$

At low levels, superoxide anion ($\cdot O_2^-$), hydroxyl radicals ($OH\cdot$), hydrogen peroxide (H_2O_2), and molecular oxygen (O_2) take part in numerous cellular processes, including cell proliferation, apoptosis, immune responses, and cell differentiation. Overproduction or inadequate removal of ROS can result in oxidative stress, leading to altered metabolism, dysregulated signal transduction events, and biomolecular damage, all of which contribute to pathological changes in cell and tissue function. Biomolecular damage that occurs as a result of elevated ROS levels is manifested as lipid peroxidation, DNA

[1]National Institute of Environmental Health Sciences, Laboratory of Molecular Toxicology, [2]Laboratory of Molecular Carcinogenesis, and [3]Laboratory of Environmental Carcinogenesis and Mutagenesis, Research Triangle Park, NC 27709.

mutations/breakage, enzyme inactivation/activation, and protein oxidation/degradation (51). This damage is often secondary to the production of reactive biomolecules (*e.g.,* hydroxynonenal and malondialdehyde) that arise as the result of ROS action (57). The action of reactive biomolecules, thus, serves to exacerbate the damaging effects of ROS.

ATTENUATION OF ROS ACTION

Antioxidants function to delay or prevent ROS-induced cellular damage, and work by reducing local oxygen concentrations, impairing chain initiation reactions, binding catalysts such as metal ions that generate ROS, and attenuating hydrogen abstraction by active radicals (65). They include low-molecular-weight compounds such as β-carotene, ascorbate, tocopherols, uric acid, the thiol-containing compound glutathione (GSH), as well as high-molecular-weight antioxidant enzymes, and proteins such as metallothionein and ferritin.

The low-molecular-weight antioxidants β-carotene (vitamin A) and ascorbate (vitamin C) attenuate the damaging effects of O_2 in lipid and nonlipid cellular compartments. Cytosolic ascorbate takes part in the continuous regeneration of tocopherol (vitamin E) (80), demonstrating the cooperative relationship between cellular antioxidant synthesis. The primary function of tocopherol is to prevent lipid peroxidation chain reactions and quench O_2 in cellular lipid compartments (12). Uric acid protects against oxidative damage by scavenging O_2, $OH^{\cdot}$, and other cellular oxidants (6). GSH, an important water-phase antioxidant and essential cofactor for antioxidant enzymes, contributes to the recycling of oxidized antioxidants, and serves as a scavenger of $^{\cdot}O_2^-$ and $OH^{\cdot}$ (65).

Major high-molecular-weight cellular antioxidant enzymes and proteins that modulate the action of ROS or their by-products include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), the thioredoxin/thioredoxin reductase system, metallothioneins, and ferritin (80). SOD (mitochondrial manganese SOD and cytoplasmic copper-zinc SOD) inactivates $^{\cdot}O_2^-$ by converting it to H_2O_2, whereas CAT inactivates H_2O_2 via its dissociation to water and oxygen. GPx, in the presence of reduced glutathione (GSH) also converts H_2O_2 to water. In the latter reaction, GSH is converted to its oxidized form (GSSG), and subsequently regenerated from GSSG by GR (19). The thioredoxin/thioredoxin reductase system quenches free radicals and functions in the recycling of oxidized ascorbate (3). Functionally, thioredoxin reductase reduces oxidized thioredoxin in the presence of NAD(P)H. Reduced thioredoxin then serves as an electron donor for thioredoxin peroxidase, which reduces H_2O_2 to water (68). Metallothioneins, a family of zinc-binding proteins, act as antioxidants. Their functions include free radical/transition metal sequestering, regulation of zinc homeostasis, and interactions with GSH (66). In general, iron (Fe^{2+}) plays a key role in antioxidant enzyme function where it acts as a catalyst during the reduction of H_2O_2 to $OH^{\cdot}$ (40). Cellular levels of Fe^{2+} are maintained at a minimum to avoid excessive production of $OH^{\cdot}$ via the Fenton reaction ($Fe^{2+} + \mathbf{H_2O_2} \rightarrow Fe^{3+} + OH^- + \mathbf{OH^{\cdot}}$). This is accomplished in part by the iron storage protein ferritin, which limits the catalytic availability of Fe^{2+} for participation in oxygen radical generation (79).

ROS-MEDIATED SKIN CANCER INDUCTION

ROS-induced DNA damage

It is well known that oxidative stress contributes to skin carcinogenesis, especially following UV irradiation (31). Some of the deleterious events that occur in cells following ROS exposure include DNA damage, cell-cycle dysregulation, DNA repair and/or replication disturbance, and gene mutations. ROS-induced DNA damage also induces the production of immunosuppressive cytokines and immune suppression that may contribute to the emergence of skin cancer (Fig. 1).

ROS-induced DNA damage includes mutations in growth regulatory genes that lead to loss of cell-cycle control and DNA repair and contribute to abnormal apoptosis. An example is the tumor suppressor p53, which regulates multiple growth inhibitory and apoptotic genes. ROS action results in loss of function mutations in the p53 gene, resulting in abnormal cell function (64). ROS, moreover, regulate p53 in a posttranslational manner via reduction or oxidation of intramolecular disulfide bonds, rendering wild-type p53 a conformational mutant, *i.e.,* producing a similar biological outcome as p53 mutations (47). Research indicates that treatment of mice with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA), UV radiation, or tumor-promoting phorbol esters induces H_2O_2 generation and the formation of oxidized bases in epidermal DNA (85) that may contribute to mutations in p53 and other genes. Events of this type are not limited to rodents as high levels of 8-hydroxy-2′-deoxyguanosine DNA adducts (8-OHdG), a by-product of $OH^{\cdot}$ attack on DNA, appear in normal human epidermis following UV irradiation (1). Other forms of DNA damage associated with ROS activity [*e.g.,* cyclobutyl pyrimidine dimers and (6-4) photoproducts] also occur in the skin of humans who have increased sensitivity to UVB radiation (81), and in organ-cultured human skin exposed to UVB radiation (55).

Antioxidants attenuate cancer induction and/or its development, indicating that ROS-induced DNA damage can be controlled pharmacologically (73). Examples include tocopherol and epigallocatechin gallate, a green tea polyphenol that protects skin from oxidative stress, both of which delay the onset of radiation-induced skin cancer in rodents (25). *In vitro*, treatment of mouse keratinocytes with ascorbic acid, Trolox (a water-soluble form of tocopherol), or selenite (a known antioxidant) results in a significant decrease in the number of detectable UVB radiation-induced 8-OHdG adducts (77). This effect is not consistent across experimental systems because ascorbate, tocopherol, or depletion of endogenous GSH lowers cellular sensitivity to UVB radiation-induced oxidative damage in immortalized HaCaT keratinocytes. However, the DNA-damaging effects of UVA radiation can be counteracted by pretreatment with the same antioxidants in HaCaT keratinocytes (39).

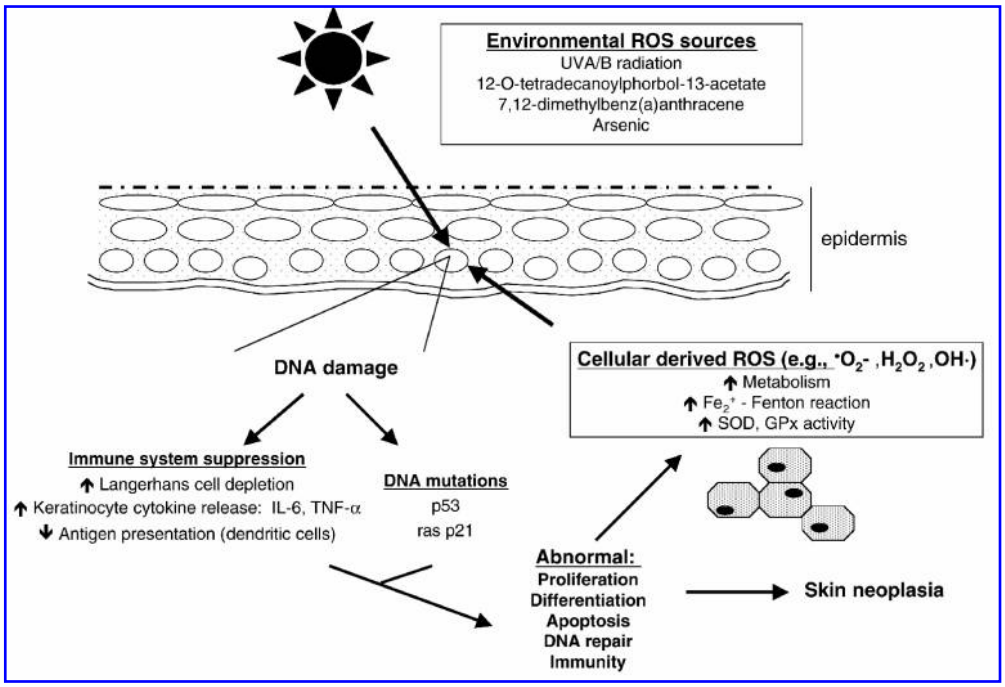

FIG. 1. DNA damage: a central event in skin cancer. DNA damage is central to altered cell proliferation, differentiation, DNA repair, cell death, and immune system function required for skin cancer development. Exogenously and endogenously derived ROS (*e.g.,* $OH^{\cdot}$ and H_2O_2) induce mutations in growth regulatory genes (*e.g.,* p21 ras, p53) and can disrupt normal immune system function. The effects of ROS result in abnormal cellular physiology that contribute to elevated ROS (*e.g.,* increased SOD activity), thus maintaining the DNA damage cycle, and potential for cancer-causing events to occur.

ROS-induced immune system modulation

Skin cancer development depends on immune surveillance breakdown mediated in part by ROS action. UV irradiation, for instance, produces a number of adverse effects on the immune system, including suppression of systemic and cutaneous immune responsiveness, and immunologic unresponsiveness to cutaneous tumors (32). ROS-induced immune modulation revolves around DNA, as it is one of the primary molecular targets associated with immune dysfunction in the skin. ROS generate DNA damage that correlates with a reduction in contact sensitization, an impaired ability of dendritic cells in the draining lymph nodes to present antigen, and the development of hapten-specific suppressor T lymphocytes (82). Evidence that DNA damage is a contributing event in immune system suppression comes from studies utilizing liposomes containing T4 endonuclease V, an enzyme that repairs cyclobutyl pyrimidine dimers (33), or photolyase, an enzyme that splits radiation-induced cyclobutyl pyrimidine dimers (83). The activity of photolyase, for example, reduces the immune suppressing effects of UV irradiation as measured by a reduction in contact hypersensitivity, impairment in antigen-presenting cell function, and the induction of transferable immune suppression (82).

Antioxidant studies demonstrate that ROS-induced immunosuppression can be modulated. For example, immune suppression following UV irradiation permits the outgrowth of developing skin tumors, an effect attenuated by tocopherol (75). In humans, high-dose tocopherol combined with ascorbate results in a reduction in radiation-induced immune suppression (20). Protection against this effect, evaluated by suppression of epidermal contact hypersensitivity in mice, also occurs following exposure to green tea polyphenol extracts (30). *In vitro*, tocopherol attenuates radiation-induced oxidative events and immune suppression in isolated epidermal cells, monitored by measuring lipid peroxidation, and lymphocyte proliferation induced by epidermal cells or purified Langerhans cells in mixed epidermal cell–lymphocyte cultures (14). GSH, similar to low-molecular-weight antioxidants, also protects against local and systemic immune suppression that may contribute to skin cancer induction by UVB radiation (74).

THE DUAL ROLE OF ROS IN PSORIASIS

Excessive generation of ROS by the immune system results in pathological changes that contribute to inflammatory disorders such as scleroderma and psoriasis (17). Psoriasis is characterized by elevated keratinocyte proliferation, abnormal keratinocyte differentiation, alterations in dermal vasculature, elevated cellular antioxidant activities, and the presence of dermal/epidermal T cells, monocytes/macrophages, and polymorphonuclear leukocytes (PMNs) within or near psoriatic lesions (16).

ROS contribute to the development and/or maintenance of psoriasis in a numbers of ways. Figure 2 depicts several

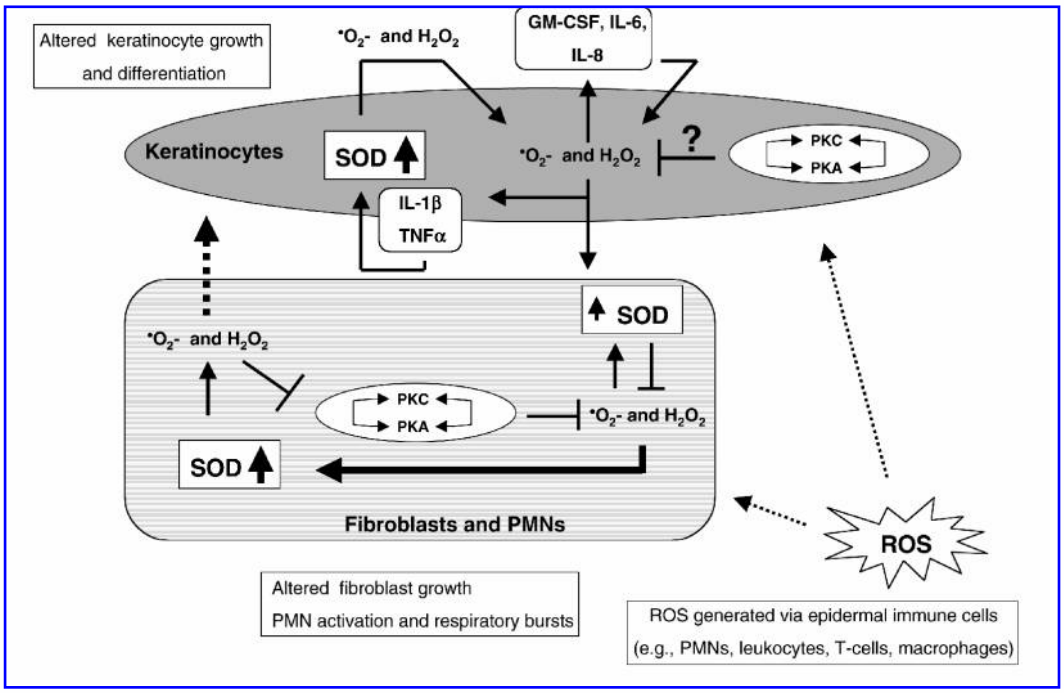

FIG. 2. Simplified model of ROS involvement in psoriasis. Cross-talk between PKC and PKA down-regulates cellular ROS (*e.g.,* $\cdot O_2^-$ and H_2O_2) generation in fibroblasts and PMNs. This effect is attenuated by elevated ROS levels, the partial result of high SOD activity, that increase keratinocyte growth and epidermal inflammation. ROS action results in both paracrine and autocrine effects on epidermal keratinocytes, fibroblasts, and PMNs. ROS and ROS-induced keratinocyte proinflammatory cytokine release enhance other events in the skin (*e.g.,* leukocyte cytokine release) that fuel continued ROS production.

cellular events, proposed to be important in psoriasis, that are influenced by ROS action. PMNs, for example, secrete $\cdot O_2^-$ and H_2O_2 during respiratory bursts that result in the elevated ROS levels within the skin (52). Altered antioxidant levels occur in a variety of cell types residing within psoriatic lesions, providing further evidence that ROS, either directly or indirectly, influence endogenous detoxification mechanisms. Elevated interleukin (IL)-1β and tumor necrosis factor-α (TNF-α)-induced SOD expression occurs in psoriatic lesions *in vivo* and in cultured human keratinocytes *in vitro*, demonstrating a relationship between proinflammatory cytokine expression and ROS level/activity (42). Under normal conditions, elevated SOD expression is a protective response mediated by cytokines released from inflammatory cells invading diseased skin. However, high or long-term SOD expression can result in the inappropriate production of ROS such as H_2O_2 in intra- and extracellular compartments (49). Indeed, TNF-α-enhanced SOD activity, $\cdot O_2^-$ generation, and H_2O_2-mediated complement activation occur in fibroblasts (71), and may contribute to inflammation and tissue damage at the site of psoriatic skin lesions.

At the molecular level, cross-talk between protein kinase C (PKC) and protein kinase A (PKA) functions in part to down-regulate respiratory bursts in PMNs and release of ROS (67). Dermal fibroblasts *in vitro* also display decreased PKA activity, measured by catalytic subunit binding, following exposure to $\cdot O_2^-$ and H_2O_2 (63). PKA activity is reduced in psoriatic fibroblasts *in vivo* (11), suggesting that ROS, at the molecular level, serve to alter fibroblast PKA activity and cyclic AMP binding. Down-regulation of PKA activity is significant because in normal fibroblasts and other cell types, elevation of intracellular cyclic AMP (*i.e.,* elevated PKA activity) is inhibitory to cell growth and division (58). Indeed, ROS stimulate cell proliferation and trigger undifferentiated keratinocytes into forming a defective stratum corneum, an event central to the development of psoriasis (69). Abnormal keratinocyte growth in psoriasis, thus, may be the partial result of elevated $\cdot O_2^-$ generated by PMNs, and psoriatic dermal fibroblasts (18).

In contrast to their pathological role in psoriasis, ROS appear to be of benefit in the therapeutic management of this disorder. Oxidative stress generation following exposure to dithranol (anthralin), a widely used antipsoriatic drug with tumor-promoting and skin-irritation properties, indirectly suggests that ROS contribute to the therapeutic effects of antipsoriatic compounds (45). Oxidative stress generation at the site of anthralin treatment also correlates with increased production of the proinflammatory cytokines granulocyte macrophage stimulating factor (GM-CSF), IL-6, IL-8, and TNF-α, all of which may enhance immune system function necessary for disease attenuation (35). Anthralin-induced cytotoxicity in primary epidermal keratinocytes, evaluated by changes in plasma membrane integrity, lysosomal function, and mitochondrial metabolic activity, indicates that $\cdot O_2^-$ and H_2O_2 contribute to the therapeutic effects of this compound (24), possibly via the loss of glucose-6-phosphate dehydrogenase activity (54). Anthralin potently inhibits human monocyte $\cdot O_2^-$ generation (53), further demonstrating that this

drug blunts respiratory bursts involved in the etiology of psoriasis.

Antioxidants, including tocopherol, ameliorate nonspecific ROS toxicity, demonstrated by the selective inhibition of erythema and hyperpigmentation that occurs following psoralen (furocoumarins) treatment of psoriasis (61). Antioxidant-attenuated toxicity may be due to tocopherol interfering with free radical formation rather than by scavenging anthralin radicals directly (60). SOD and tocopherol also reduce the damaging side effects of anthralin-induced ROS action without loss of therapeutic efficacy (34). Although antioxidants diminish the nonspecific toxicity of anthralin-induced ROS, it is unknown if these agents can impair or reverse changes in cell structure and function mediated by ROS. Partial support for the latter has been suggested by Raynaud *et al.,* who demonstrated that small molecular weight and enzymatic antioxidants attenuate PKA activity modulated by ROS (63).

MODEL SYSTEMS IN WHICH TO STUDY ROS ACTION

Animal and cell-culture models provide a powerful approach in which to elucidate the contribution of ROS to a variety of skin diseases and disorders. Some of these experimental systems have not been engineered or traditionally used to examine the role of ROS in skin; however, this should not rule out their application in research designed to elucidate the pathological effects of ROS.

In vivo *models*

The SKH-1 hairless mouse has been used extensively in antioxidant and ROS research. Studies performed by Lopez-Torres *et al.* in SKH-1 mice suggest that continuous damage to skin by free radicals occurs during the lifetime of an organism (43). In a comparative study using SKH-1 mice, it was found that human skin is more susceptible to free radical formation, an effect that might be due in part to a unique visible light chromophore found in human skin (27). The dose-response effects of acute UV irradiation on dermal/epidermal antioxidant levels including CAT, GPx, GR, ascorbate, and GSH in SKH-1 mice indicate that the antioxidant capacity of the epidermis is greater than that of dermis (70). Tocopherol blunts radiation-induced ROS action via its ability to elevate dermal SOD activity and protect epidermal GPx and SOD from depletion in this model (43). Similarly, tocopherol sorbate, α-tocopherol, and tocopherol acetate decrease free-radical formation in SKH-1 mouse skin (28). Other effects of ROS in SKH-1 mice have been evaluated, and include the induction of epidermal DNA damage (50), antioxidant effects on skin carcinogenesis (8), and epidermal immune system modulation (76).

Although the reason(s) for its increased sensitivity to ROS action has not been elucidated, the inbred SENCAR (SENsitivity to CARcinogenesis) strain of mice has been used to examine oxidant involvement and the preventative effects of antioxidants in skin tumor promotion, oxidative stress generation, and inflammation (86). For example, the flavonoid compound silymarin, derived from the milk thistle plant (*Silybum marianum*), is thought to be protective against UV radiation-induced skin cancer. Studies in SENCAR mice demonstrate the inhibitory effects of this compound on tumor promotion, oxidative stress generation, and inflammatory responses (86). Tumor promotion inhibition with other naturally occurring compounds, including nordihydroguaiaretic acid and diallyl sulfide antioxidants (5), as well as polyphenolic fractions (isolated from green tea), also has been evaluated in the SENCAR mouse model (29).

Transgenic antioxidant-deficient or -overexpressing mice provide a unique opportunity to modulate antioxidant activity *in vivo* without pharmacological manipulation. The tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induces oxidative damage to DNA and decreases the activity of several antioxidant enzymes, including GPx. Transgenic animals that express inducible GPx display pronounced GPx induction and no loss of GPx activity, as compared with non-transgenic control mice, following TPA exposure (9). Carcinogenic responses to initiation by DMBA and promotion with TPA have been evaluated in C57 transgenic mice (C57BL/6 × CBA/J) that overexpress SOD and GPx (44). In this study, overexpression of skin antioxidant enzymes leads to increased, rather than decreased, tumorigenesis in a two-stage skin carcinogenesis model, and may provide support for the prooxidant nature of cellular enzymatic antioxidants.

The Tg.AC transgenic mouse carries the coding sequence of v-Ha-ras linked to a ζ-globin promoter and an SV40 polyadenylation signal sequence (23). Tg.AC mice develop skin papillomas, dependent on v-Ha-ras transgene activity, in response to topically applied genotoxic and nongenotoxic carcinogens and tumor promoters. One study examining ROS action in Tg.AC mice indicates that inducible nitric oxide synthase, an enzyme that contributes to oxidative stress generation, is up-regulated following tumor promotion with TPA (37). Experiments designed to elucidate the effect of UV irradiation on tumorigenesis in Tg.AC mice indicate that p53 mutations or protein loss is not a common occurrence during the induction of photocarcinogenesis (78). The latter suggests that the Tg.AC mouse may be an appropriate model for skin cancers that have a ROS component, but do not display mutations in p53 [*e.g.,* arsenic-induced skin cancer (13)].

In vitro *models*

In vitro, keratinocyte, fibroblast, and melanocyte cultures are appropriate epidermal models that can be used to determine cellular and biochemical details of ROS action. For example, immortalized human HaCaT keratinocytes have been used to examine mitochondria ROS accumulation (21) and the effects of the oxidizing agents and their immune-modulating role in allergic contact dermatitis (41) and in radiation-induced antioxidant defense regulation (36). Cellular sensitivity to ROS production, effects on apoptosis, and antioxidant cell defense, following Bcl-2 overexpression, also have been evaluated in HaCaT keratinocytes (84). In immortalized mouse HEL30 keratinocytes, arsenic induces concentration- and time-dependent increases in cellular oxidative activity (15). This effect is attenuated by rotenone pretreatment, suggesting the involvement of mitochondria-derived ROS in the overexpression of growth factors that play a role

in arsenic-induced skin hyperkeratoses, cancer (15), and possibly other diseases.

Although displaying a finite life span, primary human keratinocytes, fibroblasts, and melanocytes provide realistic *in vitro* models in which to examine oxidative effects on cutaneous function. Primary human keratinocytes have been used to examine the role of oxidative stress in redox cycling (72) modulation of mitogenic, inflammatory, and apoptotic signal transduction (4). Primary human dermal fibroblast studies suggest that ROS play an active role in oxidative stress-induced connective tissue damage (10), heme oxygenase-1 induction, and its role in cellular Fe^{2+} homeostasis (2). Studies using human melanocytes demonstrate the differential sensitivity of melanocytes and melanoma cells to H_2O_2-induced oxidative stress (48), melanogenesis, and its regulation by GSH (7). The relationship between cell death and increased sensitivity to oxidative stress in vitiligo melanocytes *in vitro* (26) and imbalance in the antioxidant system in these cells (46) provide further support for ROS-mediated damage as a pathogenic event in melanocyte degeneration.

In vitro skin equivalent models have come into favor in recent years when performing studies where cell–cell interactions may be critical. Skin equivalents are prepared by culturing human keratinocytes on fibroblasts embedded in collagen gel that closely resembles human skin (38). One study examining antioxidant depletion in a skin equivalent model demonstrates that UVA and UVB irradiation depletes cellular tocopherol and ascorbate, and this correlates with detectable oxidative damage (59).

PERSPECTIVE

ROS play a dual role in the development of skin disease and disorders. Genetic alterations leading to abnormal cell function and immune suppression following ROS exposure are likely to permit the development of skin disease, such as cancer, over the lifetime of an individual.

Taken together, the evidence presented here and elsewhere in the literature demonstrates that oxidative mechanisms play a role in the pathogenesis of skin disorders and disease. Research indicates that antioxidants offer protection against the damaging effects of oxidative stress *in vitro* and *in vivo*. There is considerable interest in determining whether agents with antioxidant properties can be of benefit in preventing the onset or progression of skin pathologies in human beings. Thus, reliable data regarding the benefits of antioxidant supplementation will emerge from continued basic research, clinical, and epidemiological studies.

SUMMARY

ROS are agents that play a role in both normal and abnormal epidermal physiology. This review is an attempt to discuss briefly various aspects of excessive free radical action, targets, and attenuation in biologically relevant model systems. Oxygen radicals are generated during normal metabolism and are an integral part of cellular function (*e.g.,* immune-mediated destruction of bacteria and virus-infected cells, inhibition of tumor cell growth, and cytochrome P450-mediated chemical detoxification). The generation of ROS usually has little consequence because of a vast array of protective intracellular defenses in the skin. However, under certain circumstances, increased ROS production can overwhelm the cellular defense mechanisms used to detoxify these species.

Important reactants in cells are oxygen and its radical derivatives, including $\cdot O_2^-$, $OH\cdot$, and H_2O_2. The skin has developed a comprehensive array of defenses that prevent ROS formation or limit their damaging effects, including both enzymatic and nonenzymatic antioxidants. ROS formed within cells can oxidize biomolecules resulting in DNA damage, lipid peroxidation, and enzyme dysfunction that contribute to cellular toxicity, pathology, or death. The end result of chronic, elevated exposure to ROS is changes in cell function that contribute to skin cancer and psoriasis. The use of sensitive or transgenic animal models will help in further delineating the effects of ROS in these disorders and others.

Many questions remain to be answered concerning the physiological effects of ROS. It is anticipated that continued research will increase knowledge concerning the role of ROS in skin disease, and continue to shed light on therapeutic strategies that can be used to reduce or eliminate cutaneous pathophysiology.

ACKNOWLEDGMENTS

The authors would like to thank Kristen Geisenhoffer, Dr. James Bonner, Dr. Maria Kadiiska, Dr. Mariana Stern, and Dr. Jean Harry for their thoughtful contributions to this article.

ABBREVIATIONS

CAT, catalase; DMBA, 7,12-dimethylbenz[a]anthracene; GM-CSF, granulocyte macrophage stimulating factor; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; H_2O_2, hydrogen peroxide; IL, interleukin; $\cdot O_2^-$, superoxide anion; $OH\cdot$, hydroxyl radical; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PKA, protein kinase A; PKC, protein kinase C; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; UV, ultraviolet.

REFERENCES

1. Ahmed NU, Ueda M, Nikaido O, Osawa T, and Ichihashi M. High levels of 8-hydroxy-2′-deoxyguanosine appear in normal human epidermis after a single dose of ultraviolet radiation. *Br J Dermatol* 140: 226–231, 1999.
2. Applegate LA, Scaletta C, Panizzon R, and Frenk E. Evidence that ferritin is UV inducible in human skin: part of a putative defense mechanism. *J Invest Dermatol* 111: 159–163, 1998.

3. Arner ES and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267: 6102–6109, 2000.
4. Assefa Z, Garmyn M, Bouillon R, Merlevede W, Vandenheede JR, and Agostinis P. Differential stimulation of ERK and JNK activities by ultraviolet B irradiation and epidermal growth factor in human keratinocytes. *J Invest Dermatol* 108: 886–891, 1997.
5. Athar M, Raza H, Bickers DR, and Mukhtar H. Inhibition of benzoyl peroxide-mediated tumor promotion in 7,12-dimethylbenz(a)anthracene-initiated skin of Sencar mice by antioxidants nordihydroguaiaretic acid and diallyl sulfide. *J Invest Dermatol* 94: 162–165, 1990.
6. Becker BF, Reinholz N, Leipert B, Raschke P, Permanetter B, and Gerlach E. Role of uric acid as an endogenous radical scavenger and antioxidant. *Chest* 100 (3 Suppl): 176S–181S, 1991.
7. Benathan M and Labidi F. Cysteine-dependent 5-*S*-cysteinyldopa formation and its regulation by glutathione in normal epidermal melanocytes. *Arch Dermatol Res* 288: 697–702, 1996.
8. Berton TR, Conti CJ, Mitchell DL, Aldaz CM, Lubet RA, and Fischer SM. The effect of vitamin E acetate on ultraviolet-induced mouse skin carcinogenesis. *Mol Carcinog* 23: 175–184, 1998.
9. Bilodeau JF and Mirault ME. Increased resistance of GPx-1 transgenic mice to tumor promoter-induced loss of glutathione peroxidase activity in skin. *Int J Cancer* 80: 863–867, 1999.
10. Brenneisen P, Briviba K, Wlaschek M, Wenk J, and Scharffetter-Kochanek K. Hydrogen peroxide (H_2O_2) increases the steady-state mRNA levels of collagenase/MMP-1 in human dermal fibroblasts. *Free Radic Biol Med* 22: 515–524, 1997.
11. Brion DE, Raynaud F, Plet A, Laurent P, Leduc B, and Anderson W. Deficiency of cyclic AMP-dependent protein kinases in human psoriasis. *Proc Natl Acad Sci U S A* 83: 5272–5276, 1986.
12. Burton G and Ingold K. *Mechanisms of Antioxidant Action: Preventive and Chain-Breaking Antioxidants.* Boca Raton, FL: CRC Press, 1989, pp. 29–43.
13. Castren K, Ranki A, Welsh JA, and Vahakangas KH. Infrequent p53 mutations in arsenic-related skin lesions. *Oncol Res* 10: 475–482, 1998.
14. Clement-Lacroix P, Michel L, Moysan A, Morliere P, and Dubertret L. UVA-induced immune suppression in human skin: protective effect of vitamin E in human epidermal cells in vitro. *Br J Dermatol* 134: 77–84, 1996.
15. Corsini E, Asti L, Viviani B, Marinovich M, and Galli CL. Sodium arsenate induces overproduction of interleukin-1alpha in murine keratinocytes: role of mitochondria. *J Invest Dermatol* 113: 760–765, 1999.
16. Das UN, Vijaykumar K, Madhavi N, Suryaprabha P, Sravankumar G, Ramesh G, Koratkar R, Sagar PS, and Padma M. Psoriasis: current concepts and new approaches to therapy. *Med Hypotheses* 38: 56–62, 1992.
17. De Simone C, De Sole P, Di Mario G, Venier A, Cerimele D, and Serri F. Reactive oxygen species production in circulating polymorphonuclear leukocytes in psoriasis. *Acta Derm Venereol Suppl (Stockh)* 146: 50–52, 1989.
18. Er-raki A, Charveron M, and Bonafe JL. Increased superoxide anion production in dermal fibroblasts of psoriatic patients. *Skin Pharmacol* 6: 253–258, 1993.
19. Fuchs J. *Oxidative Injury in Dermatopathology.* Berlin, Heidelberg: Springer-Verlag, 1992, pp. 48–57.
20. Fuchs J and Packer L. Antioxidant protection from solar-simulated radiation-induced suppression of contact hypersensitivity to the recall antigen nickel sulfate in human skin. *Free Radic Biol Med* 27: 422–427, 1999.
21. Gniadecki R, Thorn T, Vicanova J, Petersen A, and Wulf HC. Role of mitochondria in ultraviolet-induced oxidative stress. *J Cell Biochem* 80: 216–222, 2000.
22. Halliwell B and Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect* 102 (Suppl 10): 5–12, 1994.
23. Hansen LA, Spalding JW, French JE, and Tennant RW. A transgenic mouse model (TG.AC) for skin carcinogenesis: inducible transgene expression as a second critical event. *Prog Clin Biol Res* 391: 223–235, 1995.
24. Hsieh GC and Acosta D. Dithranol-induced cytotoxicity in primary cultures of rat epidermal keratinocytes. I. The role of reactive oxygen species. *Toxicol Appl Pharmacol* 107: 16–26, 1991.
25. Ichihashi M, Ahmed NU, Budiyanto A, Wu A, Bito T, Ueda M, and Osawa T. Preventive effect of antioxidant on ultraviolet-induced skin cancer in mice. *J Dermatol Sci* 23 (Suppl 1): S45–S50, 2000.
26. Jimbow K, Chen H, Park JS, and Thomas PD. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. *Br J Dermatol* 144: 55–65, 2001.
27. Jurkiewicz BA and Buettner GR. EPR detection of free radicals in UV-irradiated skin: mouse versus human. *Photochem Photobiol* 64: 918–922, 1996.
28. Jurkiewicz BA, Bissett DL, and Buettner GR. Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. *J Invest Dermatol* 104: 484–488, 1995.
29. Katiyar SK, Agarwal R, Ekker S, Wood GS, and Mukhtar H. Protection against 12-*O*-tetradecanoylphorbol-13-acetate-caused inflammation in SENCAR mouse ear skin by polyphenolic fraction isolated from green tea. *Carcinogenesis* 14: 361–365, 1993.
30. Katiyar SK, Elmets CA, Agarwal R, and Mukhtar H. Protection against ultraviolet-B radiation-induced local and systemic suppression of contact hypersensitivity and edema responses in C3H/HeN mice by green tea polyphenols. *Photochem Photobiol* 62: 855–861, 1995.
31. Kensler TW and Trush MA. Role of oxygen radicals in tumor promotion. *Environ Mutagen* 6: 593–616, 1984.
32. Kripke ML and Fisher MS. Immunologic parameters of ultraviolet carcinogenesis. *J Natl Cancer Inst* 57: 211–215, 1976.
33. Kripke ML, Cox PA, Bucana C, Vink AA, Alas L, and Yarosh DB. Role of DNA damage in local suppression of contact hypersensitivity in mice by UV radiation. *Exp Dermatol* 5: 173–180, 1996.
34. Lange RW, Germolec DR, Foley JF, and Luster MI. Antioxidants attenuate anthralin-induced skin inflammation in BALB/c mice: role of specific proinflammatory cytokines. *J Leukoc Biol* 64: 170–176, 1998.

35. Lange RW, Hayden PJ, Chignell CF, and Luster MI. Anthralin stimulates keratinocyte-derived proinflammatory cytokines via generation of reactive oxygen species. *Inflamm Res* 47: 174–181, 1998.
36. Leccia MT, Richard MJ, Joanny-Crisci F, and Beani JC. UV-A1 cytotoxicity and antioxidant defence in keratinocytes and fibroblasts. *Eur J Dermatol* 8: 478–482, 1998.
37. Lee BM, Kim HS, and Jeohn GH. Inducible nitric oxide synthase mRNA is upregulated in skin tumors of v-ha-ras transgenic TG-AC mice treated with 12-*O*-tetradecanoylphorbol-13- acetate. *Biol Pharm Bull* 23: 826–829, 2000.
38. Lee DY, Ahn HT, and Cho KH. A new skin equivalent model: dermal substrate that combines de-epidermized dermis with fibroblast-populated collagen matrix. *J Dermatol Sci* 23: 132–137, 2000.
39. Lehmann J, Pollet D, Peker S, Steinkraus V, and Hoppe U. Kinetics of DNA strand breaks and protection by antioxidants in UVA- or UVB-irradiated HaCaT keratinocytes using the single cell gel electrophoresis assay. *Mutat Res* 407: 97–108, 1998.
40. Liochev SI and Fridovich I. The role of $O_2^{\cdot -}$ in the production of $HO^{\cdot}$: in vitro and in vivo. *Free Radic Biol Med* 16: 29–33, 1994.
41. Little MC, Metcalfe RA, Haycock JW, Healy J, Gawkrodger DJ, and MacNeil S. The participation of proliferative keratinocytes in the preimmune response to sensitizing agents. *Br J Dermatol* 138: 45–56, 1998.
42. Lontz W, Sirsjo A, Liu W, Lindberg M, Rollman O, and Torma H. Increased mRNA expression of manganese superoxide dismutase in psoriasis skin lesions and in cultured human keratinocytes exposed to IL-1 beta and TNF-alpha. *Free Radic Biol Med* 18: 349–355, 1995.
43. Lopez-Torres M, Thiele JJ, Shindo Y, Han D, and Packer L. Topical application of alpha-tocopherol modulates the antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. *Br J Dermatol* 138: 207–215, 1998.
44. Lu YP, Lou YR, Yen P, Newmark HL, Mirochnitchenko OI, Inouye M, and Huang MT. Enhanced skin carcinogenesis in transgenic mice with high expression of glutathione peroxidase or both glutathione peroxidase and superoxide dismutase. *Cancer Res* 57: 1468–1474, 1997.
45. Mader K, Bacic G, and Swartz HM. In vivo detection of anthralin-derived free radicals in the skin of hairless mice by low-frequency electron paramagnetic resonance spectroscopy. *J Invest Dermatol* 104: 514–517, 1995.
46. Maresca V, Roccella M, Roccella F, Camera E, Del Porto G, Passi S, Grammatico P, and Picardo M. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J Invest Dermatol* 109: 310–313, 1997.
47. Maxwell SA and Roth JA. Posttranslational regulation of p53 tumor suppressor protein function. *Crit Rev Oncog* 5: 23–57, 1994.
48. Meyskens FL, Chau HV, Tohidian N, and Buckmeier J. Luminol-enhanced chemiluminescent response of human melanocytes and melanoma cells to hydrogen peroxide stress. *Pigment Cell Res* 10: 184–189, 1997.
49. Midorikawa K and Kawanishi S. Superoxide dismutases enhance H_2O_2-induced DNA damage and alter its site specificity. *FEBS Lett* 495: 187–190, 2001.
50. Mitchell DL, Byrom M, Chiarello S, and Lowery MG. Attenuation of DNA damage in the dermis and epidermis of the albino hairless mouse by chronic exposure to ultraviolet-A and -B radiation. *Photochem Photobiol* 73: 83–89, 2001.
51. Miyachi Y. [Biochemistry of the physiopathologic and clinical aspects of free radicals in skin diseases]. *Nippon Rinsho* 46: 2252–2256, 1988.
52. Miyachi Y and Niwa Y. Effects of psoriatic sera on the generation of oxygen intermediates by normal polymorphonuclear leucocytes. *Arch Dermatol Res* 275: 23–26, 1983.
53. Mrowietz U, Falsafi M, Schroder JM, and Christophers E. Inhibition of human monocyte functions by anthralin. *Br J Dermatol* 127: 382–386, 1992.
54. Muller K, Seidel M, Braun C, Zieresi K, and Wiegrebe W. Dithranol, glucose-6-phosphate dehydrogenase inhibition and active oxygen species. *Arzneimittelforschung* 41: 1176–1181, 1991.
55. Muramatsu T, Kobayashi N, Tada H, Yamaji M, Shirai T, Mori T, and Ohnishi T. Induction and repair of UVB-induced cyclobutane pyrimidine dimers and (6-4) photoproducts in organ-cultured normal human skin. *Arch Dermatol Res* 284: 232–237, 1992.
56. Nachbar F and Korting HC. The role of vitamin E in normal and damaged skin. *J Mol Med* 73: 7–17, 1995.
57. Parola M, Bellomo G, Robino G, Barrera G, and Dianzani MU. 4-Hydroxynonenal as a biological signal: molecular basis and pathophysiological implications. *Antioxid Redox Signal* 1: 255–284, 1999.
58. Pastan IH, Johnson GS, and Anderson WB. Role of cyclic nucleotides in growth control. *Annu Rev Biochem* 44: 491–522, 1975.
59. Podda M, Traber MG, Weber C, Yan LJ, and Packer L. UV-irradiation depletes antioxidants and causes oxidative damage in a model of human skin. *Free Radic Biol Med* 24: 55–65, 1998.
60. Popov I and Lewin G. A deficient function of the antioxidative system of the organism as an aetiopathogenetic factor in psoriasis. *Med Hypotheses* 35: 229–236, 1991.
61. Potapenko AY and Kyagova AA. The application of antioxidants in investigations and optimization of photochemotherapy. *Membr Cell Biol* 12: 269–278, 1998.
62. Powers SK and Hamilton K. Antioxidants and exercise. *Clin Sports Med* 18: 525–536, 1999.
63. Raynaud F, Evain-Brion D, Gerbaud P, Marciano D, Gorin I, Liapi C, and Anderson WB. Oxidative modulation of cyclic AMP-dependent protein kinase in human fibroblasts: possible role in psoriasis. *Free Radic Biol Med* 22: 623–632, 1997.
64. Renzing J, Hansen S, and Lane DP. Oxidative stress is involved in the UV activation of p53. *J Cell Sci* 109: 1105–1112, 1996.
65. Rice-Evans CA and Burdon RH. *Free Radical Damage and its Control.* Amsterdam: Elsevier Science B.V., 1994, pp. 113–114.
66. Sato M and Bremner I. Oxygen free radicals and metallothionein. *Free Radic Biol Med* 14: 325–337, 1993.

67. Savitha G and Salimath BP. Cross-talk between protein kinase C and protein kinase A down-regulates the respiratory burst in polymorphonuclear leukocytes. *Cell Signal* 5: 107–117, 1993.
68. Schallreuter KU and Wood JM. Thioredoxin reductase—its role in epidermal redox status. *J Photochem Photobiol B* 64: 179–184, 2001.
69. Shilov VN and Sergienko VI. Oxidative stress in keratinocytes as an etiopathogenetic factor of psoriasis. *Bull Exp Biol Med* 129: 364–369, 2000.
70. Shindo Y, Witt E, and Packer L. Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J Invest Dermatol* 100: 260–265, 1993.
71. Shingu M, Nonaka S, Nobunaga M, and Ahamadzadeh N. Possible role of H_2O_2-mediated complement activation and cytokines-mediated fibroblasts superoxide generation on skin inflammation. *Dermatologica* 179 (Suppl 1): 107–112, 1989.
72. Shvedova AA, Kommineni C, Jeffries BA, Castranova V, Tyurina YY, Tyurin VA, Serbinova EA, Fabisiak JP, and Kagan VE. Redox cycling of phenol induces oxidative stress in human epidermal keratinocytes. *J Invest Dermatol* 114: 354–364, 2000.
73. Slaga TJ. Inhibition of the induction of cancer by antioxidants. *Adv Exp Med Biol* 369: 167–174, 1995.
74. Steenvoorden DP and Beijersbergen van Henegouwen G. Glutathione ethylester protects against local and systemic suppression of contact hypersensitivity induced by ultraviolet B radiation in mice. *Radiat Res* 150: 292–297, 1998.
75. Steenvoorden DP and Beijersbergen van Henegouwen G. Protection against UV-induced systemic immunosuppression in mice by a single topical application of the antioxidant vitamins C and E. *Int J Radiat Biol* 75: 747–755, 1999.
76. Steerenberg PA, Garssen J, Dortant P, Hollman PC, Alink GM, Dekker M, Bueno-de-Mesquita HB, and Van Loveren H. Protection of UV-induced suppression of skin contact hypersensitivity: a common feature of flavonoids after oral administration? *Photochem Photobiol* 67: 456–461, 1998.
77. Stewart MS, Cameron GS, and Pence BC. Antioxidant nutrients protect against UVB-induced oxidative damage to DNA of mouse keratinocytes in culture. *J Invest Dermatol* 106: 1086–1089, 1996.
78. Trempus CS, Mahler JF, Ananthaswamy HN, Loughlin SM, French JE, and Tennant RW. Photocarcinogenesis and susceptibility to UV radiation in the v-Ha-ras transgenic Tg.AC mouse. *J Invest Dermatol* 111: 445–451, 1998.
79. Tsuji Y, Ayaki H, Whitman SP, Morrow CS, Torti SV, and Torti FM. Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. *Mol Cell Biol* 20: 5818–5827, 2000.
80. Tyrrell RM. The molecular and cellular pathology of solar ultraviolet radiation. *Mol Aspects Med* 15: 1–77, 1994.
81. Ueda M, Matsunaga T, Bito T, Nikaido O, and Ichihashi M. Higher cyclobutane pyrimidine dimer and (6-4) photoproduct yields in epidermis of normal humans with increased sensitivity to ultraviolet B radiation. *Photodermatol Photoimmunol Photomed* 12: 22–26, 1996.
82. Vink AA, Strickland FM, Bucana C, Cox PA, Roza L, Yarosh DB, and Kripke ML. Localization of DNA damage and its role in altered antigen-presenting cell function in ultraviolet-irradiated mice. *J Exp Med* 183: 1491–1500, 1996.
83. Vink AA, Moodycliffe AM, Shreedhar V, Ullrich SE, Roza L, Yarosh DB, and Kripke ML. The inhibition of antigen-presenting activity of dendritic cells resulting from UV irradiation of murine skin is restored by in vitro photorepair of cyclobutane pyrimidine dimers. *Proc Natl Acad Sci U S A* 94: 5255–5260, 1997.
84. Virgili F, Santini MP, Canali R, Polakowska RR, Haake A, and Perozzi G. Bcl-2 overexpression in the HaCaT cell line is associated with a different membrane fatty acid composition and sensitivity to oxidative stress. *Free Radic Biol Med* 24: 93–101, 1998.
85. Wei L, Wei H, and Frenkel K. Sensitivity to tumor promotion of SENCAR and C57BL/6J mice correlates with oxidative events and DNA damage. *Carcinogenesis* 14: 841–847, 1993.
86. Zhao J, Lahiri-Chatterjee M, Sharma Y, and Agarwal R. Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. *Carcinogenesis* 21: 811–816, 2000.

Address reprint requests to:
Dr. Dori R. Germolec
111 T.W. Alexander Drive
Bldg. 101, Rm. C-140, MD C1-04
P.O. Box 12233
Research Triangle Park, NC 27709

E-mail: germolec@niehs.nih.gov

Received for publication April 19, 2002; accepted April 27, 2002.

This article has been cited by:

1. Ali Murat Ceyhan, Vahide Baysal Akkaya, #eyma Celik Güleçol, Betül Mermi Ceyhan, Fehmi Özgüner, WenChieh Chen. 2012. Protective effects of #-glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats. *Archives of Dermatological Research* **304**:7, 521-527. [CrossRef]
2. Daniel Wright, Mariagioia Zampagni, Elisa Evangelisti, Simona Conti, Giampiero D'Adamio, Andrea Goti, Matteo Becatti, Claudia Fiorillo, Niccolò Taddei, Cristina Cecchi, Gianfranco Liguri. 2012. Protective Properties of Novel S- Acyl-Glutathione Thioesters Against Ultraviolet-induced Oxidative Stress. *Photochemistry and Photobiology* n/a-n/a. [CrossRef]
3. WonBong Lim , JiSun Kim , ChaeGwang Lim , SangWoo Kim , SangMi Jeon , Sandeep Karna , MinSung Cho , HongRan Choi , OkJoon Kim . 2012. Effect of 635 nm Light-Emitting Diode Irradiation on Intracellular Superoxide Anion Scavenging Independent of the Cellular Enzymatic Antioxidant System. *Photomedicine and Laser Surgery* **30**:8, 451-459. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Ashley R. Murray, Elena Kisin, Alfred Inman, Shih-Houng Young, Mamoun Muhammed, Terrance Burks, Abdusalam Uheida, Alexey Tkach, Micah Waltz, Vincent Castranova, Bengt Fadeel, Valerian E. Kagan, Jim E. Riviere, Nancy Monteiro-Riviere, Anna A. Shvedova. 2012. Oxidative Stress and Dermal Toxicity of Iron Oxide Nanoparticles In Vitro. *Cell Biochemistry and Biophysics* . [CrossRef]
5. Meital Portugal-Cohen, Liran Horev, Claas Ruffer, Gerrit Schlippe, Werner Voss, Ze'evi Ma'or, Miriam Oron, Yoram Soroka, Marina Fruši#-Zlotkin, Yoram Milner, Ron Kohen. 2012. Non-invasive skin biomarkers quantification of psoriasis and atopic dermatitis: Cytokines, antioxidants and psoriatic skin auto-fluorescence. *Biomedicine & Pharmacotherapy* **66**:4, 293-299. [CrossRef]
6. Souren Paul, Heung Sop Shin, Sun Chul Kang. 2012. Inhibition of inflammations and macrophage activation by ginsenoside-Re isolated from Korean ginseng (Panax ginseng C.A. Meyer). *Food and Chemical Toxicology* **50**:5, 1354-1361. [CrossRef]
7. Guei-Sheung Liu, Hitesh Peshavariya, Masayoshi Higuchi, Alison C. Brewer, Catherine W.T. Chang, Elsa C. Chan, Gregory J. Dusting. 2012. Microphthalmia-associated transcription factor modulates expression of NADPH oxidase type 4: A negative regulator of melanogenesis. *Free Radical Biology and Medicine* **52**:9, 1835-1843. [CrossRef]
8. Thomas G Polefka, Thomas A Meyer, Patricia P Agin, Robert J Bianchini. 2012. Cutaneous oxidative stress. *Journal of Cosmetic Dermatology* **11**:1, 55-64. [CrossRef]
9. Stefan Hübner, Athina Efthymiadis. 2012. Recent progress in histochemistry and cell biology. *Histochemistry and Cell Biology* . [CrossRef]
10. R.M. Clegg1.24 Fluorescence and FRET: Theoretical Concepts 101 592-617. [CrossRef]
11. O. Arican, P. Ozturk, E.B. Kurutas, V. Unsal. 2011. Status of oxidative stress on lesional skin surface of plantar warts. *Journal of the European Academy of Dermatology and Venereology* no-no. [CrossRef]
12. Dong Hyun Kim, Dashlkhumbe Byamba, Wen H. Wu, Tae-Gyun Kim, Min-Geol Lee. 2011. Different characteristics of reactive oxygen species production by human keratinocyte cell line cells in response to allergens and irritants. *Experimental Dermatology* no-no. [CrossRef]
13. Adriana Filip, Doina Daicoviciu, Simona Clichici, Pompei Bolfa, Cornel Catoi, Ioana Baldea, Laura Bolojan, Diana Olteanu, Adriana Muresan, I.D. Postescu. 2011. The effects of grape seeds polyphenols on SKH-1 mice skin irradiated with multiple doses of UV-B. *Journal of Photochemistry and Photobiology B: Biology* . [CrossRef]
14. April W. Armstrong, Stephanie V. Voyles, Ehrin J. Armstrong, Erin N. Fuller, John C. Rutledge. 2011. Angiogenesis and oxidative stress: Common mechanisms linking psoriasis with atherosclerosis. *Journal of Dermatological Science* **63**:1, 1-9. [CrossRef]

15. Zhe Jian, Kai Li, Ling Liu, Ying Zhang, Zhou Zhou, Chunying Li, Tianwen Gao. 2011. Heme Oxygenase-1 Protects Human Melanocytes from H2O2-Induced Oxidative Stress via the Nrf2-ARE Pathway. *Journal of Investigative Dermatology* **131**:7, 1420-1427. [CrossRef]
16. Jian-ping Wang, Ya-ming Zhou, Yu-jie Ye, Xian-mei Shang, Ya-ling Cai, Chao-mei Xiong, Yun-xia Wu, Hai-xing Xu. 2011. Topical anti-inflammatory and analgesic activity of kirenol isolated from Siegesbeckia orientalis. *Journal of Ethnopharmacology* . [CrossRef]
17. Suvendu Kumar Behera, Umesh Dimri, Shanker Kumar Singh, Ranjan Kumar Mohanta. 2011. The curative and antioxidative efficiency of ivermectin and ivermectin + vitamin E-selenium treatment on canine Sarcoptes scabiei infestation. *Veterinary Research Communications* **35**:4, 237-244. [CrossRef]
18. Wanakee J. Carr, Rebecca E. Oberley-Deegan, Yuping Zhang, Christopher C. Oberley, Larry W. Oberley, Martine Dunnwald. 2011. Antioxidant proteins and reactive oxygen species are decreased in a murine epidermal side population with stem cell-like characteristics. *Histochemistry and Cell Biology* **135**:3, 293-304. [CrossRef]
19. A Damasiewicz-Bodzek, T Wielkoszy#ski. 2011. Advanced protein glycation in psoriasis. *Journal of the European Academy of Dermatology and Venereology* no-no. [CrossRef]
20. M. A. Babizhayev, G. M. Nikolayev, J. G. Nikolayeva, Y. E. Yegorov. 2011. A survey and analysis of the role of molecular chaperone proteins and imidazole-containing dipeptide-based compounds as molecular escorts into the skin during stress, injury, water structuring and other types of cutaneous pathophysiology. *International Journal of Cosmetic Science* **33**:1, 1-16. [CrossRef]
21. Mostafa A. Saleh, Osman M. Mahran, M. Bassam Al-Salahy. 2011. Circulating oxidative stress status in dromedary camels infested with sarcoptic mange. *Veterinary Research Communications* **35**:1, 35-45. [CrossRef]
22. Kyung Su Yoon, Xue Zhu Huang, Yang Suk Yoon, Soo-Ki Kim, Soon Bong Song, Byung Soo Chang, Dong Heui Kim, Kyu Jae Lee. 2011. Histological Study on the Effect of Electrolyzed Reduced Water-Bathing on UVB Radiation-Induced Skin Injury in Hairless Mice. *Biological & Pharmaceutical Bulletin* **34**:11, 1671-1677. [CrossRef]
23. Taesook Yoon, Do Yeon Lee, A Yeong Lee, Goya Choi, Byung Kil Choo, Ho Kyoung Kim. 2010. Anti-inflammatory effects of Glehnia littoralis extract in acute and chronic cutaneous inflammation. *Immunopharmacology and Immunotoxicology* **32**:4, 663-670. [CrossRef]
24. Mark A Babizhayev, Gennady M Nikolayev, Juliana G Nikolayeva, Yegor E Yegorov. 2010. Biologic Activities of Molecular Chaperones and Pharmacologic Chaperone Imidazole-Containing Dipeptide-Based Compounds: Natural Skin Care Help and the Ultimate Challenge. Implication for Adaptive Responses in the Skin. *American Journal of Therapeutics* 1. [CrossRef]
25. Hui Xu, Pin-ru Wu, Zheng-yu Shen, Xiang-dong Chen. 2010. Chemical analysis of Hericium erinaceum polysaccharides and effect of the polysaccharides on derma antioxidant enzymes, MMP-1 and TIMP-1 activities. *International Journal of Biological Macromolecules* **47**:1, 33-36. [CrossRef]
26. Arjen Joosse, Esther De Vries, Casper H. Van Eijck, Alexander M. M. Eggermont, Tamar Nijsten, Jan Willem W. Coebergh. 2010. Reactive oxygen species and melanoma: an explanation for gender differences in survival?. *Pigment Cell & Melanoma Research* **23**:3, 352-364. [CrossRef]
27. Isabel F. Almeida, Paulo C. Costa, M. Fernanda Bahia. 2010. Evaluation of Functional Stability and Batch-to-Batch Reproducibility of a Castanea sativa Leaf Extract with Antioxidant Activity. *AAPS PharmSciTech* **11**:1, 120-125. [CrossRef]
28. Sharif M. Al-Reza, Jung In Yoon, Hyo Jung Kim, Jong-Sang Kim, Sun Chul Kang. 2010. Anti-inflammatory activity of seed essential oil from Zizyphus jujuba. *Food and Chemical Toxicology* **48**:2, 639-643. [CrossRef]
29. A. T. Grazul-Bilska, J. J. Bilski, D. A. Redmer, L. P. Reynolds, K. M. Abdullah, A. Abdullah. 2009. Antioxidant capacity of 3D human skin EpiDerm TM model: effects of skin moisturizers. *International Journal of Cosmetic Science* **31**:3, 201-208. [CrossRef]

30. Do Yeon Lee, Goya Choi, Taesook Yoon, Myeong Sook Cheon, Byung Kil Choo, Ho Kyoung Kim. 2009. Anti-inflammatory activity of Chrysanthemum indicum extract in acute and chronic cutaneous inflammation. *Journal of Ethnopharmacology* **123**:1, 149-154. [CrossRef]
31. Aaron K Holley, Daret K St Clair. 2009. Watching the watcher: regulation of p53 by mitochondria. *Future Oncology* **5**:1, 117-130. [CrossRef]
32. D LEE, B CHOO, T YOON, M CHEON, H LEE, A LEE, H KIM. 2009. Anti-inflammatory effects of Asparagus cochinchinensis extract in acute and chronic cutaneous inflammation. *Journal of Ethnopharmacology* **121**:1, 28-34. [CrossRef]
33. Peter Bai, Csaba Heged#s, Éva Szabó, László Gyüre, Edina Bakondi, Attila Brunyánszki, Szabolcs Gergely, Csaba Szabó, László Virág. 2009. Poly(ADP-Ribose) Polymerase Mediates Inflammation in a Mouse Model of Contact Hypersensitivity. *Journal of Investigative Dermatology* **129**:1, 234-238. [CrossRef]
34. Chen N Young, Jay I Koepke, Laura J Terlecky, Michael S Borkin, Savoy L Boyd, Stanley R Terlecky. 2008. Reactive Oxygen Species in Tumor Necrosis Factor-#-Activated Primary Human Keratinocytes: Implications for Psoriasis and Inflammatory Skin Disease. *Journal of Investigative Dermatology* **128**:11, 2606-2614. [CrossRef]
35. Vittorio Calabrese, Stella Calafato, Eduardo Puleo, Carolin Cornelius, Maria Sapienza, Pierfrancesco Morganti, Cesare Mancuso. 2008. Redox regulation of cellular stress response by ferulic acid ethyl ester in human dermal fibroblasts: role of vitagenes. *Clinics in Dermatology* **26**:4, 358-363. [CrossRef]
36. U DIMRI, R RANJAN, N KUMAR, M SHARMA, D SWARUP, B SHARMA, M KATARIA. 2008. Changes in oxidative stress indices, zinc and copper concentrations in blood in canine demodicosis. *Veterinary Parasitology* **154**:1-2, 98-102. [CrossRef]
37. Ali Karaman, Cihangir Alia#ao#lu, #braham Pirim. 2008. Sister chromatid exchange analysis in patients with psoriasis. *Experimental Dermatology* **17**:6, 524-529. [CrossRef]
38. Eduard P.A. Van Wijk , Rainer Lüdtke , Roeland Van Wijk . 2008. Differential Effects of Relaxation Techniques on Ultraweak Photon Emission. *The Journal of Alternative and Complementary Medicine* **14**:3, 241-250. [Abstract] [Full Text PDF] [Full Text PDF with Links]
39. Sabrina Narayanan, Andreas Hùnerbein, Melkamu Getie, Andreas Jäckel, Reinhard H. H. Neubert. 2007. Scavenging properties of metronidazole on free oxygen radicals in a skin lipid model system. *Journal of Pharmacy and Pharmacology* **59**:8, 1125-1130. [CrossRef]
40. Meital Portugal, Vivian Barak, Isaac Ginsburg, Ron Kohen. 2007. Interplay among oxidants, antioxidants, and cytokines in skin disorders: Present status and future considerations. *Biomedicine & Pharmacotherapy* **61**:7, 412-422. [CrossRef]
41. Anna Wojas-Pelc, Janusz Marcinkiewicz. 2007. What is a role of haeme oxygenase-1 in psoriasis? Current concepts of pathogenesis. *International Journal of Experimental Pathology* **88**:2, 95-102. [CrossRef]
42. Paola Chiarugi , Francesca Buricchi . 2007. Protein Tyrosine Phosphorylation and Reversible Oxidation: Two Cross-Talking Posttranslation Modifications. *Antioxidants & Redox Signaling* **9**:1, 1-24. [Abstract] [Full Text PDF] [Full Text PDF with Links]
43. H HA, Y KIM, Z RYOO, T KIM. 2006. Inhibition of the TPA-induced cutaneous inflammation and hyperplasia by EC-SOD. *Biochemical and Biophysical Research Communications* **348**:2, 450-458. [CrossRef]
44. A RUFFIENCISZAK, P GROS, M COMTAT, A SCHMITT, E QUESTEL, C CASAS, D REDOULES. 2006. Exploration of the global antioxidant capacity of the stratum corneum by cyclic voltammetry. *Journal of Pharmaceutical and Biomedical Analysis* **40**:1, 162-167. [CrossRef]
45. Yu-Ying He, Jian-Li Huang, Michelle L. Block, Jau-Shyong Hong, Colin F. Chignell. 2005. Role of Phagocyte Oxidase in UVA-Induced Oxidative Stress and Apoptosis in Keratinocytes. *Journal of Investigative Dermatology* **125**:3, 560-566. [CrossRef]

46. Christopher G. De Vry, Marybeth Valdez, Mirella Lazarov, Evelyn Muhr, Roland Buelow, Timothy Fong, Suhasini Iyer. 2005. Topical Application of A Novel Immunomodulatory Peptide, RDP58, Reduces Skin Inflammation in the Phorbol Ester-Induced Dermatitis Model. *Journal of Investigative Dermatology* **125**:3, 473-481. [CrossRef]
47. Fabio Virgili, R Ambra, R Canali, O GulatiPycnogenol in Cancer Chemoprevention **20041296**, . [CrossRef]
48. Ryan Moseley, Joanna R. Hilton, Rachel J. Waddington, Keith G. Harding, Phil Stephens, David W. Thomas. 2004. Comparison of oxidative stress biomarker profiles between acute and chronic wound environments. *Wound Repair and Regeneration* **12**:4, 419-429. [CrossRef]
49. Kiymet Baz, M. Y. Burak Cimen, Aysin Kokturk, Gonul Aslan, Guliz Ikizoglu, D. Deniz Demirseren, Arzu Kanik, Ugur Atik. 2004. Plasma reactive oxygen species activity and antioxidant potential levels in rosacea patients: correlation with seropositivity to Helicobacter pylori. *International Journal of Dermatology* **43**:7, 494-497. [CrossRef]
50. Yaxiong Xie, Kevin J. Trouba, Jie Liu, Michael P. Waalkes, Dori R. Germolec. 2004. Biokinetics and Subchronic Toxic Effects of Oral Arsenite, Arsenate, Monomethylarsonic Acid, and Dimethylarsinic Acid in v-Ha-ras Transgenic (Tg.AC) Mice. *Environmental Health Perspectives* **112**:12, 1255-1263. [CrossRef]
51. Zhe Huang, Yuichiro Senoh, Shinya Katoh, Nobuhiko Miwa. 2004. Preventive effects of a water-soluble derivative of chroman moiety of vitamin E on lipid hydroperoxide-induced cell injuries and DNA cleavages through repressions of oxidative stress in the cytoplasm of human keratinocytes. *Journal of Cellular Biochemistry* **92**:3, 425-435. [CrossRef]
52. Sumio Yano, Nelly Arroyo, Noriko Yano. 2004. Catalase binds Grb2 in tumor cells when stimulated with serum or ligands for integrin receptors. *Free Radical Biology and Medicine* **36**:12, 1542-1554. [CrossRef]
53. Marta Wrona, Malgorzata Ró#anowska, Tadeusz Sarna. 2004. Zeaxanthin in combination with ascorbic acid or #-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radical Biology and Medicine* **36**:9, 1094-1101. [CrossRef]
54. K. Trouba, A. Nyska, M. Styblo, D. Dunson, L. Lomnitski, S. Grossman, G. Moser, A. Suttie, R. Patterson, F. Walton, D. GermolecEffect of antioxidants on the papilloma response and liver glutathione modulation mediated by arsenic in Tg.AC transgenic mice 283-293. [CrossRef]