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Review Article

Antioxidants in dermocosmetology: from the laboratory to clinical application

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Pour faciliter la lecture, les parties essentielles en ce qui concerne la coenzyme Q10 NOVA ©SOL (hydrophile et micellaire) et la société Aquanova sont colorées en vert page 5

Abstract

Oxygen situated in cutaneous cells can be activated by light. This makes the integumentary apparatus particularly vulnerable to oxidative damage and is responsible for the immediate cutaneous damage that is the basis of late phenomena, such as photo-induced ageing and tumours. Thus, the cosmetic industry has undertaken research and development into antioxidant-based products able to protect the skin from the effect of pro-oxidizing noxae.

This review re-examines both antioxidants suitable for dermatological application and skin care products with antioxidant capacity, as well as the laboratory methods used to evaluate the effects and *in vivo* efficacy of antioxidants.

Keywords: antioxidants, cosmetic products, free radicals, photo-induced damage

Introduction

Cosmetological research has increasingly focused on processes leading to the formation of anatomical–functional damage to the skin, identified with ageing. At the same time, every possible means to counteract the injurious effects have been evaluated. Great interest in this topic has been aroused by the study of substances able to prevent cutaneous damage by free radicals; these substances are currently termed antioxidants.[1,2]

Direct tests of their efficacy have been performed mainly in the laboratory, with a series of models based on human and animal cells subjected to various types of oxidative stress.[3] Under these experimental conditions, many substances, with more or less complex chemical structures, have been found to possess antiradical activity and have been introduced onto the market as anti-ageing products. However, in many cases, there is little evidence of the their efficacy *in vivo*, which is a basic prerequisite for these products to be used successfully to prevent skin damage.

Therefore, we have examined this topic in the light of recent evidence, particularly concerning the causes of cutaneous oxidative stress, the mechanisms leading to the formation of structural damage, the active principles that might be used to prevent such damage and those for which a certain clinical efficacy has been demonstrated.

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Oxidative stress and protective systems

Free radicals can be defined as molecules or fragments of molecules, capable of independent existence, containing one or more unpaired electrons in their external orbitals. They tend to react very easily with various types of biomolecules, to acquire another electron and stabilize the orbital. Examples of free radicals are the superoxide anion (O_2^-), hydroxyl ($OH^\bullet$) and nitric oxide ($NO^\bullet$). The term 'reactive oxygen species' (ROS) indicates not only free radicals based on oxygen, but also some non-radical by-products of oxygen, like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$).

Organisms constantly produce free radicals by different mechanisms.[4] Incomplete reduction of oxygen in the mitochondrial electron transport chain releases superoxide anions into the cytoplasm.[5] In cells with phagocytic activity, such as neutrophils and macrophages, great quantities of ROS are formed during oxidative metabolism.[6] Under these conditions, the production of ROS is controlled by neutralization systems, in concert with the

ongoing biological processes. It is only when ROS evade the control systems that free radicals can give rise to toxic phenomena.

The cytotoxic action of free radicals is opposed by a double defence system, constituted by some enzymes with specific activity and by simple molecules with low molecular mass normally introduced into the organism in food. The former are represented mainly by catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, and the latter mainly by ascorbic acid and tocopherol.

Superoxide dismutase accelerates the speed of dismutation of the superoxide anion into hydrogen peroxide by ≈ 1000×. This prevents the two oxidized species from coexisting and thus generating the hydroxyl radical ($OH^{\bullet}$), an extremely reactive organic species. Catalase is able to reduce hydrogen peroxide, a reaction catalysed by glutathione peroxidase, which can also regenerate ascorbic acid from dehydroascorbic acid.[7,8]

Human skin possesses all the above-mentioned systems with antioxidant activity.[9] It also has numerous enzymes that regulate redox reactions, with the additional activity of regenerating the antioxidant defence systems.[10]

Generators of free radicals and skin damage

The formation of free radicals is a risk for all tissues, including skin. Indeed, skin is particularly exposed to oxidative damage: in addition to being supplied with oxygen from the blood, it also exchanges this element with the external environment. Moreover, oxygen situated in cutaneous cells can be activated by light and this makes the integumentary apparatus particularly vulnerable to oxidative damage. Among the stimuli able to cause oxidative stress in the skin are inflammatory agents that attract macrophages and neutrophils, cells specialized in generating and releasing highly reactive oxidizing species.

Oxidative stress can also be an important pathogenetic factor in damage due to ischaemia, which can occur during dermosurgical operations or in vasculopathic cutaneous pathologies.[11]

Another agent with oxidative activity in the skin is ozone.[12] Ozone is normally present in the atmosphere, where it has an important role in filtering short-wavelength UV radiation. However, it can be toxic at ground level as a product of photochemical smog at concentrations of 0.1–0.5 p.p.m., representing a serious threat to urban air quality.[13,14]

Nevertheless, photo-exposure is by far the most important condition for the formation of free radicals in the skin: ultraviolet radiation (UV), particularly the band between 290 and 400 nm, is the main cause of oxidative stress.[15] Its ability to penetrate into the skin varies according to the wavelength: radiation of 300 nm (UVB) exhausts its energy in the epidermis, whereas radiation of 350 nm (UVA) penetrates more deeply. Thus, whereas the principal site of action of UVB is the epidermis, UVA acts mainly in the dermis.[16]

It has now been demonstrated that UV rays are able to generate ROS *in vivo*, which are then responsible for a large quantity and variety of tissue damage.[17] In skin irradiated with UV, one frequently finds lipoperoxidation and the formation of sunburn cells, a marker of UVB-damaged epidermis that can be modulated by substances with scavenger activity. Moreover, and this is particularly important, UV irradiation of the skin leads to compromise of the antioxidant defences. In particular, under the effect of UV radiation, the activities of superoxide dismutase, catalase and glutathione peroxidase decrease and the levels of antioxidant vitamins are greatly reduced; these events favour the formation of additional oxidative stress.[18] Finally, it should be remembered that UV rays can activate photosensitizing substances, both endogenous, e.g. porphyrins and flavins[19] and exogenous, e.g. some drugs[3] responsible for the formation of highly reactive ROS like singlet oxygen.

It is reasonable to believe that the well-known biological effects manifested after photo-exposure, particularly erythema and hyperpigmentation, are the direct consequence of oxidative stress induced by UV radiation. A similar cause–effect relationship is not easily demonstrated for biological damage that appears later. However, if one accepts the principle that photo-ageing and cutaneous tumours are also related to photo-exposure, one can infer that they are caused by pathogenesis from oxidative stress.[20]

Skin provides an excellent model for the study of ageing: it is easily accessible and the constitutive elements of its tissues, including keratinocytes, melanocytes and fibroblasts, can be cultivated and used for cellular and molecular investigations. Cutaneous senescence follows the general rules of ageing, basically due to a kind of biological 'pace-maker' that controls the duration of the life of each living being (the so-called intrinsic ageing). However, studies using the above-mentioned methods have shown that cutaneous senescence is strongly accelerated by the action of external environmental factors, especially solar radiation, which strongly influences the senescence of regions exposed to light. UV rays are mainly responsible for cutaneous ageing; they alter the cells and the amorphous and fibrillary structures of the skin by various mechanisms, such as the formation of bonds that alter the spatial structure of proteins and nucleic acids, membrane damage and the accumulation of metabolites. All these events are mediated by the formation of free radicals, believed to be fundamental factors in the process of photo-ageing.

Polyunsaturated fatty acids, an important constituent of the horny layer, are particularly susceptible to attack by free radicals through lipid peroxidation. In addition, free radicals induce depolymerization of polysaccharides, e.g. hyaluronic acid, which play an important role in defining the biomechanical properties of the skin. The cholesterol molecules in biomembranes also undergo self-oxidation, giving rise to the formation of oxysterols, responsible for altered membrane fluidity.

The most striking structural modification of photo-ageing is the accumulation of elastotic material. This is due to the direct effects of UV radiation on transcriptional activation of the elastin gene, as shown by a transgenic mouse model of photo-ageing. The release of cytokines by photostimulation causes an inflammatory state and aggravates this process.[21] Dermal elastosis leads to the formation of skin that is thinner, rougher and less elastic. The epidermal alterations, essentially due to depletion of the lipid portion of the horny layer, lead to the phenomenon known as dry skin, causing the appearance of a fine surface rugosity.[22]

Photo-ageing is usually the precursor of carcinogenesis and some factors invoked to explain the role of light in the pathogenesis of senescence can also be used to explain the onset of tumours. Indeed, the appearance of cutaneous tumours, particularly squamous cell carcinomas, is proportional to the amount of photo-exposure, i.e. the quantity of electromagnetic energy absorbed. A presumptive role of free radicals is suggested by the finding that cutaneous tumours are less frequent in subjects fed a diet rich in antioxidants, as well as in animals treated with topical applications based on vitamins C and E. It is also believed that free radicals play an important role in the onset of melanoma.[23] However, it should be remembered that the onset and progression of cutaneous tumours may also involve other factors able to influence the mechanism of carcinogenesis.

Evaluation of oxidative stress

Free radicals have only a fleeting existence *in vivo* and thus their direct measurement is not easy. Nevertheless, they are able to activate a series of signals resulting in the transcription of biologically important proteins. Moreover, as a consequence of their action, numerous biomolecules undergo irreversible modifications that can be used as biomarkers of oxidative stress.

Electron paramagnetic resonance is the most selective technique for the direct measurement of free radicals and the skin is currently the only organ that can be explored with sufficient sensitivity by this method.[24,25]

It can detect and identify the superoxide anion, hydroxyl and other radical species, which all have a very short half-life. This limitation has favoured the development of methods based on the spin trapping method. This can be defined as a chemical reaction in which the radical is bound to a molecule that acts as a trap for the radical species, thus allowing its measurement. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) is the substance most widely used as a spin trap in biological systems, but it has not yet been employed in clinical dermatology.[26,27]

Stimuli able to generate free radicals and the consequent variations of ROS levels not only cause damage to biomolecules, but can also interfere with the pathways leading to signal transduction. Therefore, even modest variations of ROS levels can induce complex responses which, through gene modulation, culminate in the production of proteins that can significantly modify the biology of the cell.

Among the most important mediators of the cell's response to oxidative stress are mitogen-activated protein kinases (MAPKinases), a family of enzymes that control and coordinate a great variety of extra- and intracellular signals.[28]

Nuclear factor κB (NF-κB) is a multiprotein complex that controls the transcription of a large number of genes critical for the regulation of various cellular processes. Activation of NF-κB could be the response to stress induced by a great variety of stimuli, such as pharmacological agents, ozone and solar radiation.[29]

Another transcription factor whose regulation can be influenced by the redox state of the cell is AP-1 complex, composed of two subunits: c-*fos* and c-*jun*.[30] Under the stimulus of UVB radiation, they can increase their expression,[31] codifying proteins able to protect the cell from oxidative damage.[32]

Most of the molecules involved in the mechanisms of signal transduction can be labelled using immunocytochemical methods. However, none of these methods allows specific identification of the biochemical event resulting from the stressful stimulus and none has great clinical importance.[33]

One of the most important phenomena mediated by signal transduction and activated by oxidative stress is apoptosis, defined as a natural programmed pathway of cell death, of crucial importance for the development and homeostasis of many tissues. Apoptosis plays an important role in the elimination of damaged cells and is believed to be the central mechanism of cellular differentiation in constantly renewed tissues like the epidermis. Oxidative stress is a trigger factor able to induce apoptosis in various pathological and therapeutic conditions, particularly during treatment with UV radiation, alone or associated with photosensitizers.[34] Evaluation of the percentage of apoptotic cells can be used as a reference value

to estimate the oxidative stress in appropriate experimental conditions.[35]

Free radicals can leave irreversible signs in many biomolecules, which can be used as biomarkers of oxidative stress. The biomarkers induced by free radicals can be detected and visualized directly in tissues and isolated cells through histochemical and immunohistochemical reactions. Lipid peroxidation of polyunsaturated fatty acids gives rise to a vast range of aldehyde and carbonyl compounds; some of them, like malonyldialdehyde, diffuse rapidly in aqueous media, whereas other lipophilic compounds remain in the lipid phase. Other products, like α,β-unsaturated aldehydes, show a certain reactivity to the amino acid residues of the protein component. These products can be identified with histochemical reactions, e.g. periodic acid Schiff, or with alternative procedures.[36] Oxidative damage to proteins can cause the loss of protein-SH groups, which can be detected by means of fluorescent labelling of tissues or single cells.[37]

Antioxidants suitable for dermatological applications

For effective use in dermatological conditions, an antioxidant must have certain properties: it must not show local or systemic toxicity; it must reach high concentrations in the cutaneous structures, particularly those situated more externally; and it must be able to protect the skin from oxidizing noxae. In addition to these properties, substances for topical use must have physicochemical characteristics that allow their incorporation into skin care products, they must be stable once included in the final product and they must have organolectic characteristics that provide good compliance. The list of products that might have these characteristics is rather long and it is not easy to evaluate all of them in detail.

First on this list is vitamin E, a liposoluble substance that exists in nature in eight different forms. Many studies have demonstrated its undisputed antioxidant capacity, but they have also revealed some aspects that merit reflection.[38–40] Administered orally to healthy volunteers irradiated with UV rays, vitamin E alone does not have protective activity,[41] although it does show this effect when associated with ascorbic acid.[42] This suggests a synergistic action of these two antioxidants.[43] In reality, during lipid peroxidation α-tocopherol is oxidized to tocopheryl radical, a process made reversible by the addition of ascorbic acid, which can regenerate α-tocopherol from its oxidized form.

Ascorbic acid, widely distributed in the vegetable kingdom, is continually used by our bodies, where it is present in the form of a reserve in the adrenal glands, available for moments of emergency. Its antioxidant power against both immediate and late photodamage is still not completely clear when it is used alone.[44] Instead, there is convincing evidence of its role as a support of other antioxidant substances. In this regard, it should be remembered that the antioxidant effect of ascorbic acid is strictly dependent on the characteristics of the formulation, particularly concentration and pH.[45] This is important because ascorbic acid is extremely transitory, being destroyed quickly by heat, exposure to air and light. In addition to its antioxidant properties, ascorbic acid has pro-oxidizing effects whose biological importance has yet to be demonstrated.[46]

Carotenoids constitute another category of antioxidants of potential use in dermatology. They are represented by ≈ 600 compounds, chemically defined as tetraterpenoids, 100 of which are present in a variety of foods that form an integral part of our daily diet.[47–49] Carotenoids are efficient quenchers of singlet oxygen, but they are also involved in other antioxidant reactions. The ability to neutralize ROS and free radicals is not equal for all the members of this class, but varies according to their reduction potential: it is maximal for lycopene, followed by beta-carotene, zeaxanthin, lutein, canthaxanthin and astaxanthin.[50]

A potentially interesting active principle with antioxidant activity is lipoic acid, or thioctic acid, a molecule long known to be a cofactor of enzymatic reactions in important metabolic processes, particularly in oxidative decarboxylation of alpha-ketoacids.[51,52] One of the most beneficial effects of both alpha lipoic acid and DHLA is the ability to regenerate other essential antioxidants such as vitamins C and E, coenzyme Q10 and glutathione.[53]

The antioxidant characteristics of melatonin have been demonstrated in recent years. It is a very active scavenger, able to donate an electron and to form a cationic indolic radical. It is able to neutralize superoxide anion and singlet oxygen, but mainly hydroxyl radicals, which are produced under the influence of UV radiation.[54]

The processes of ageing and photo-ageing may be partly due to a decline in the levels of the endogenous cellular antioxidant coenzyme Q10, which is able to significantly suppress the expression of collagenase in human dermal fibroblasts following UVA irradiation. Coenzyme Q10 is also able to penetrate the viable layers of the epidermis and reduce the level of oxidation.[55]

The spectrum of antioxidant substances for use in skin protection also includes a long series of active principles, often with a polyphenolic structure, extracted from various plant species. Sometimes these active principles have been identified as single chemically defined compounds, whereas at other times they have been obtained as extracts consisting of mixtures of substances. The most representative of these antioxidant substances are: mixtures of

polyphenols extracted from green tea (epicatechine, epigallocatechine, epicatechine-3-gallate, epigallocatechine-3-gallate);[56] resveratrol, a polyphenol derived from European grape *Vitis vinifera* and present in the plant as an antifungal agent;[57] compounds derived from ginger, which have shown chemopreventive activity inhibiting epidermal oedema and hyperplasia caused by the application of 12-*o*-tetradecanoylphorbol-13-acetate (TPA);[58] and curcumoids, which are able to reduce the effects of UVA radiation and the application of TPA.[59]

Skin care products with antioxidant capacity

In recent years, the cosmetics market has been enriched by numerous skin care products accompanied by advertising claims centred on their antioxidant activity. These products, containing substances with antiradical activity, were created mainly to satisfy expectations of treatment and prevention of photo-ageing.[60] Their topical use is believed to be particularly effective; when applied on the skin surface, the antioxidant compounds concentrate first in the horny layer, which is the structure most exposed to oxidative stress.[61] In most of these products, the antioxidant capacity has been entrusted not to a single substance, but to an association of different principles.

Active principles with antioxidant activity have also been employed to potentiate products developed for other purposes: formulations (e.g. moisturizing creams, solar filters, anti-edemagenic gels) for use in cosmetic situations in which a possible inflammatory component might be counteracted by an antiradical substance.[62]

Unfortunately, despite the general belief in their usefulness, there is little clinical evidence of the efficacy of many products of this type. In other words, although correctly formulated on the basis of studies conducted on animal models and *in vitro*, many cosmetics labelled as antioxidants lack clear proof of their effectiveness in humans, acquired through use tests or suitable experimental designs.

The use test, carried out in conditions of the usual application of a given cosmetic, is the ideal method to ascertain the efficacy of the product. However, this procedure is difficult to perform because of the difficulty in enrolling subjects, the duration of the method (often incompatible with the needs of the manufacturers) and the relatively high costs. For these reasons, less complex protocols are now preferred, which use instruments able to provide biophysical parameters of skin functions.[63]

The most obvious cutaneous phenomenon resulting from oxidative stress is erythema, which can be evoked experimentally by chemical and physical means. The latter, particularly UV radiation, are particularly potent in inducing erythematous reactions varying in extension and intensity. These reactions can be quantified with great precision by instruments that measure redness[64] or the amount of capillary blood flow.[65]

With this model, important information has recently been acquired concerning the ability of some antioxidant agents to prevent photodamage *in vivo*. It has been found that α-tocopherol in a 2% alcohol solution can significantly inhibit the erythematous response to UV stimulation; this inhibition can be attributed to an antioxidant mechanism, because the product lacks a screening effect.[66] When applied before UV exposure, this vitamin can also reduce oedema, the formation of sunburn cells, lipid peroxidation, the formation of DNA adducts, immunosuppression and the formation of UVA-induced sensitizing substances. It has also been shown that topical application of vitamin E can reduce some late effects of UV radiation, like the formation of wrinkles and the incidence of cutaneous tumours.[67] Some esters of vitamin E (acetate, succinate, linoleate) have shown a certain ability to reduce cutaneous damage induced by UV rays. However, α-tocopherol has a more pronounced photoprotective effect than its esters, which must be hydrolysed during cutaneous absorption in order to show antioxidant activity; these metabolic aspects have still not been completely clarified.[68] The ability of α-tocopherol to counteract UV damage *in vivo* seems to be confirmed by the depletion of α-tocopherol in the horny layer after exposure to UV rays.[69]

Unlike α-tocopherol, ascorbic acid, applied as such, has only modest antioxidant effects. In a study of pig skin, vitamin C was found to be effective only when applied at high concentrations in a suitable vehicle.[70] In this regard, the use of ascorbic acid in the form of an ester, with greater antioxidant activity, appears more promising. The modest photoprotective effect of topically applied vitamin C can be explained by its instability and ease of oxidation in aqueous vehicles.[71] Vitamin C can be protected from degradation by incorporation in suitable vehicles. Moreover, the lipophilic by-products, like the palmityl and succinyl esters, could be promising compounds able to provide more lasting photoprotection than vitamin C proper.[72]

A substance widely used as an antioxidant principle in skin care products is lipoic acid, whose biochemical properties were discussed in the preceding section. At present, however, there is still a lack of convincing evidence of its clinical efficacy.

In contrast, the antioxidant activity of melatonin is well documented; it has been shown *in vivo* by studies of experimentally induced erythematous reactions in healthy volunteers. Applied on the skin before irradiation with UVB, melatonin was found to prevent the formation of erythema; this did not occur or was only minimal when the substance was applied after irradiation of the skin.[73]

The antioxidant effect of melatonin is potentiated by the presence of vitamins C and E.

Another category of antioxidants able to prevent photodamage *in vivo* is that of carotenoids. Indeed, lycopene has proved to be very active, with a high reduction potential.[74] This substance is able to protect the skin from the effects of UVB rays, especially when used in association with ascorbic acid and α-tocopherol. This confirms the hypothesis, based on laboratory data, that carotenoids work as quenchers of vitamin E, in turn being repaired by the action of ascorbic acid, which being hydrosoluble can be excreted by the organism.[75]

Conclusions

The cosmetics market offers consumers many hydrosoluble or liposoluble active principles for protection of the skin from oxidant stimuli. By this mechanism, the products should prevent photo-ageing and maintain the skin in a cosmetically pleasing condition. Unfortunately, the advertising claims for these products are not always backed by experimental evidence of their efficacy in normal conditions of use.

Recently, methods and instruments have been developed to measure *in vivo* the antiradical activity of finished products through the study of recent events after photoexposure. Tests of finished products are particularly important because they allow evaluation of the true efficacy of the formulation under study, independent of the activity of the single substances used. Cosmetic manufacturers should be encouraged to conduct such tests, which would permit them to select the most reliable products and would also promote research in cosmetology.

References

1 Pugliese PT. The skin's antioxidant systems. *Dermatol Nurs* 1998; **10**: 401–16.
2 Tebbe B. Relevance of oral supplementation with antioxidants for prevention and treatment of skin disorders. *Skin Pharmacol Appl Skin Physiol* 2001; **14**: 296–302.
3 Prior RL, Cao G. *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Radical Biol Med* 1999; **27**: 1173–81.
4 Ricquier D, Bouillaud F. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J Physiol* 2000; **529**: 3–10.
5 Stohs SJ. The role of free radicals in toxicity and disease. *J Basic Clin Physiol Pharmacol* 1995; **6**: 205–28.
6 Godin C, Caprani A, Dufaux J, Flaud P. Interactions between neutrophils and endothelial cells. *J Cell Sci* 1993; **106**: 441–51.
7 Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol* 1997; **82**: 291–5.
8 Steenvoorden DP, van Henegouwen GM. The use of endogenous antioxidants to improve photoprotection. *J Photochem Photobiol* 1997; **41**: 1–10.
9 Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol* 1994; **102**: 122–4.
10 Kehrer JP, Lund LG. Cellular reducing equivalents and oxidative stress. *Free Radical Biol Med* 1994; **17**: 65–75.
11 Melikian V, Laverson S, Zawacki B. Oxygen-derived free radical inhibition in the healing of experimental zone-of-stasis burns. *J Trauma* 1987; **27**: 151–4.
12 Weber SU, Han N, Packer L. Ozone: an emerging oxidative stressor to skin. *Curr Probl Dermatol* 2001; **29**: 52–61.
13 Mustafa MG. Biochemical basis of ozone toxicity. *Free Radical Biol Med* 1990; **9**: 245–65.
14 Thiele JJ, Podda M, Packer L. Tropospheric ozone: an emerging environmental stress to skin to skin. *Biol Chem* 1997; **378**: 1299–305.
15 Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. *J Photochem Photobiol* 2001; **63**: 88–102.
16 Kawanishi S, Hiraku Y, Oikawa S. Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat Res* 2001; **488**: 65–76.
17 Black HS. Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. *Photochem Photobiol* 1987; **46**: 213–21.
18 Shindo Y, Witt E, Packer L. Antioxidant defense mechanisms in murine epidermis and dermis and their responses to ultraviolet light. *J Invest Dermatol* 1993; **100**: 260–5.
19 Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. *J Photochem Photobiol* 1992; **14**: 105–24.
20 Gasparro FP, Mitchnick M, Nash JF. A review of sunscreen safety and efficacy. *Photochem Photobiol* 1998; **68**: 243–56.
21 Saliou C, Kitazawa M, McLaughlin L, Yang JP, Lodge JK, Tetsuka T, Iwasaki K, Cillard J, Okamoto T, Packer L. Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B activation in a human keratinocyte cell line. *Free Radical Biol Med* 1999; **26**: 174–83.
22 Berneburg M, Plettenberg H, Krutmann J. Photoaging of human skin. *Photodermatol Photoimmunol Photomed* 2000; **16**: 239–44.
23 Setlow RB. Spectral regions contributing to melanoma: a personal view. *J Invest Dermatol* 1999; **4**: 46–9.
24 Lange BA, Buettner GR. Electron paramagnetic resonance detection of free radicals in UV-irradiated human and mouse skin. *Curr Probl Dermatol* 2001; **29**: 18–25.
25 Mizushima J, Kawasaki Y, Kitano T, Sakamoto K, Kawashima M, Cooke R, Maibach HI. Electron paramagnetic resonance study utilizing stripping method on normal human stratum corneum. *Skin Res Technol* 2000; **6**: 108–11.

26 Fuchs J, Herrling T, Groth N. Detection of free radicals in skin: a review of the literature and new developments. *Curr Probl Dermatol* 2001; **29**: 1–17.

27 Togashi H, Shinzawa H, Matsuo T, Takeda Y, Takahashi T, Aoyama M, Oikawa K, Kamada H. Analysis of hepatic oxidative stress status by electron spin resonance spectroscopy and imaging. *Free Radical Biol Med* 2000; **28**: 846–53.

28 Peus D, Pittelkow MR. Reactive oxygen species as mediators of UVB-induced mitogen-activated protein kinase activation in keratinocytes. *Curr Probl Dermatol* 2001; **29**: 114–27.

29 Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells. *Free Radical Res Commun* 1992; **17**: 221–37.

30 Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1991; **1072**: 129–57.

31 Garmyn M, Degreef H. Suppression of UVB-induced c-*fos* and c-*jun* expression in human keratinocytes by N-acetylcysteine. *J Photochem Photobiol B* 1997; **37**: 125–30.

32 Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997; **337**: 1419–28.

33 Poynter ME, Janssen-Heininger YM, Buder-Hoffmann S, Taatjes DJ, Mossman BT. Measurement of oxidant-induced signal transduction proteins using cell imaging. *Free Radical Biol Med* 1999; **27**: 1164–72.

34 Godar DE. Light and death: photons and apoptosis. *J Invest Dermatol Symp Proc* 1999; **4**: 17–23.

35 Anselmi C, Ettorre A, Andreassi M, Centini M, Neri P, Di Stefano A. *In vitro* induction of apoptosis vs. necrosis by widely used preservatives: 2-phenoxyethanol, a mixture of isothiazolinones, imidazolidinyl urea and 1,2-pentanediol. *Biochem Pharmacol* 2002; **63**: 437–53.

36 Frank J, Pompella A, Biesalski HK. Histochemical visualization of oxidant stress. *Free Radical Biol Med* 2000; **29**: 1096–105.

37 Pompella A, Cambiaggi C, Dominici S, Paolicchi A, Tongiani R, Comporti M. Single-cell investigation by laser scanning confocal microscopy of cytochemical alterations resulting from extracellular oxidant challenge. *Histochem Cell Biol* 1996; **105**: 173–8.

38 Fuchs J, Milbradt R. Antioxidant inhibition of skin inflammation induced by reactive oxidants: evaluation of the redox couple dihydrolipoate/lipoate. *Skin Pharmacol* 1994; **7**: 278–84.

39 Kozachenko AI, Gurevich SM, Nagler LG. Effect of ascorbate and alpha-tocopherol on resistance of beta-carotene to oxidation. *Bull Exp Biol Med* 2000; **130**: 661–4.

40 Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr* 1995; **62**: 1315–21.

41 Werninghaus K, Meydani M, Bhawan J, Margolis R, Blumberg JB, Gilchrest BA. Evaluation of the photoprotective effect of oral vitamin E supplementation. *Arch Dermatol* 1994; **130**: 1257–61.

42 Eberlein-Konig B, Placzek M, Przybilla B. Protective effect against sunburn of combined systemic ascorbic acid (vitamin C) and D-alpha-tocopherol (vitamin E). *J Am Acad Dermatol* 1998; **38**: 45–8.

43 Fuchs J, Kern H. Modulation of UV-light-induced skin inflammation by D-alpha-tocopherol and L-ascorbic acid: a clinical study using solar simulated radiation. *Free Radical Biol Med* 1998; **25**: 1006–12.

44 Podda M, Grundmann-Kollmann M. Low molecular weight antioxidants and their role in skin ageing. *Clin Exp Dermatol* 2001; **26**: 578–82.

45 Pinnell SR, Yang H, Omar M, Monteiro-Riviere N, DeBuys HV, Walker LC, Wang Y, Levine M. Topical L-ascorbic acid: percutaneous absorption studies. *Dermatol Surg* 2000; **27**: 137–42.

46 Halliwell B. Vitamin C: antioxidant or pro-oxidant *in vivo*? *Free Radical Res* 1996; **25**: 439–54.

47 Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, McInerney J. An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. *Am J Clin Nutr* 2002; **75**: 79–86.

48 Bohm F, Edge R, Burke M, Truscott TG. Dietary uptake of lycopene protects human cells from singlet oxygen and nitrogen dioxide – ROS components from cigarette smoke. *J Photochem Photobiol B* 2001; **64**: 176–8.

49 Curran-Celentano J, Hammond BR Jr, Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am J Clin Nutr* 2001; **74**: 796–802.

50 Burke M, Edge R, Land EJ, McGarvey DJ, Truscott TG. One-electron reduction potentials of dietary carotenoid radical cations in aqueous micellar environments. *FEBS Lett* 2001; **500**: 132–6.

51 Scott BC, Aruoma OI, Evans PJ, O'Neill C, Van der Vliet A, Cross CE, Tritschler H, Halliwell B. Lipoic and dihydrolipoic acids as antioxidants. A critical evaluation. *Free Radical Res* 1994; **20**: 119–33.

52 Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radical Biol Med* 1995; **19**: 227–50.

53 Fuchs J. Potentials and limitations of the natural antioxidants RRR-alpha-tocopherol, L-ascorbic acid and beta-carotene in cutaneous photoprotection. *Free Radical Biol Med* 1998; **25**: 848–73.

54 Fischer TW, Scholz G, Knoll B, Hipler UC, Elsner P. Melatonin reduces UV-induced reactive oxygen species in a dose-dependent manner in IL-3-stimulated leukocytes. *J Pineal Res* 2001; **31**: 39–45.

55 Hoppe U, Bergemann J, Diembeck W, Ennen J, Gohla S, Harris I, Jacob J, Kielholz J, Mei W, Pollet D, Schachtschabel D, Sauermann G, Schreiner V, Stab F, Steckel F. Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors* 1999; **9**: 371–8.

56 Ahmad N, Mukhtar H. Cutaneous photochemoprotection by green tea: a brief review. *Skin Pharmacol Appl Skin Physiol* 2001; **14**: 69–76.

57 Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; **275**: 218–20.

58 Katiyar SK, Agarwal R, Mukhtar H. Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of *Zingiber officinale* rhizome. *Cancer Res* 1996; **56**: 1023–30.

59 Ishizaki C, Oguro T, Yoshida T, Wen CQ, Sueki H, Iijima M. Enhancing effect of ultraviolet A on ornithine decarboxylase induction and dermatitis evoked by 12-*o*-tetradecanoylphorbol-13-acetate and its inhibition by curcumin in mouse skin. *Dermatology* 1996; **193**: 311–17.

60 Lupo MP. Antioxidants and vitamins in cosmetics. *Clin Dermatol* 2001; **19**: 467–73.

61 Dreher F, Maibach H. Protective effects of topical antioxidants in humans. *Curr Probl Dermatol* 2001; **29**: 157–64.

62 Perricone NV. The photoprotective and anti-inflammatory effects of topical ascorbyl palmitate. *J Ger Dermatol* 1993; **1**: 5–10.

63 Jackson EM. Assessing the bioactivity of cosmetic products and ingredients. *Skin Pharmacol Appl Skin Physiol* 1999; **12**: 125–31.

64 Andreassi L, Flori L. Practical applications of cutaneous colorimetry. *Clin Dermatol* 1995; **13**: 369–73.

65 Zuang V, Rona C, Archer G, Berardesca E. Detection of skin irritation potential of cosmetics by non-invasive measurements. *Skin Pharmacol Appl Skin Physiol* 2000; **13**: 358–71.

66 Dreher F, Gabard B, Schwindt DA, Maibach HI. Topical melatonin in combination with vitamins E and C protects skin from ultraviolet-induced erythema: a human study *in vivo*. *Br J Dermatol* 1998; **139**: 332–9.

67 Thiele JJ, Dreher F, Packer L. Antioxidant defence systems in skin. In: P Elsner, H Maibach, A Rougier, eds. *Drug Vs Cosmetics: Cosmeceuticals?* New York: Marcel Dekker, 2000: pp. 145–87.

68 Alberts DS, Goldman R, Xu MJ, Dorr RT, Quinn J, Welch K, Guillen-Rodriguez J, Aickin M, Peng YM, Loescher L, Gensler H. Disposition and metabolism of topically administered alpha-tocopherol acetate: a common ingredient of commercially available sunscreens and cosmetics. *Nutr Cancer* 1996; **26**: 193–201.

69 Thiele JJ, Traber MG, Packer L. Depletion of human stratum corneum vitamin E: an early and sensitive in vivo marker of UV induced photo-oxidation. *J Invest Dermatol* 1998; **110**: 756–61.

70 Darr D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 1992; **127**: 247–53.

71 Austria R, Semenzato A, Bettero A. Stability of vitamin C derivatives in solution and topical formulations. *J Pharm Biomed Anal* 1997; **15**: 795–801.

72 Kobayashi S, Takehana M, Itoh S, Ogata E. Protective effect of magnesium-L-ascorbyl-2 phosphate against skin damage induced by UVB irradiation. *Photochem Photobiol* 1996; **64**: 224–8.

73 Bangha E, Elsner P, Kistler GS. Suppression of UV-induced erythema by topical treatment with melatonin (*N*-acetyl-5-methoxytryptamine). Influence of the application time point. *Dermatology* 1997; **195**: 248–52.

74 Black HS, Lambert CR. Radical reactions of carotenoids and potential influence on UV carcinogenesis. *Curr Probl Dermatol* 2001; **29**: 140–56.

75 Stanghellini E, Andreassi M, Ettorre A, Di Stefano A, Andreassi L. Instrumental evaluation of topical antioxidant activity. Nutri-Cosme-Ceuticals a challenge for the future? International Multidisciplinary Symposium Rome February 2002.