

TOPICAL AND SYSTEMIC PHOTOPROTECTION FOR A BETTER SKIN-UMBRELLA

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Summary

In this review we will outline the most frequent molecular damages inflicted by ultra-violet radiation via singlet Oxygen, as well as direct and indirect DNA damages. The current interpretation of the mechanisms involved in some of the clinical effects consequent to exposure of human skin to ultraviolet radiation will be discussed.

Protection offered by stratum corneum and pigmentation as well as individual differences in the capability to perform protein and DNA repair will be discussed. Emphasis will be brought on recent results relative to the parameters susceptible to affect the clinical effects of ultraviolet radiation, such as phototype and ethnicity, since data seem to indicate that ultraviolet radiation damages DNA in the decreasing order: Caucasians > Asians > African-Americans > Latinos.

The short-term clinical consequences of exposure to UV radiation will be discussed, with particular attention to the suppression of contact hypersensitivity and delayed type hypersensitivity and to the means to avoid the immune depression, or to restore the immune response.

Recent technologies to protect/restore the immune response, such as the topical application of sunscreens with specific absorption spectrum, together with metal chelators, DNA repairing enzymes or the systemic administration of specific carotenes, will be evoked.

Riassunto

In questo articolo verranno presentati i piu' frequenti danni molecolari inflitti direttamente o indirettamente dalla radiazione UV mediante assorbimento immediato o generazione di ossigeno singoletto o di altre specie reattive dell'ossigeno.

Verranno prese in considerazione le azioni protettive di fattori naturali come lo spessore dello strato corneo o l'intensita' della pigmentazione, con particolare attenzione a recenti risultati che sembrano indicare che fattori etnici possano giocare un ruolo nella protezione del DNA, poiche' la suscettibilita' al danno del DNA sembra decrescere in vari gruppi etnici nell'ordine:

Caucasici > asiatici > afro-americani > nativi dell'America Latina

Quest' articolo discuterà gli effetti clinici a breve termine dell'esposizione all'UV, con attenzione particolare alla soppressione dell'ipersensibilità di contatto e della ipersensibilità ritardata. Alcune metodiche per evitare la depressione immunitaria cutanea o per restaurarne l'azione, verranno discusse. Verranno menzionate alcune recenti tecnologie quali per esempio l'uso di schermi solari con spettri d'assorbimento ben definiti, in combinazione con agenti chelatanti, enzimi di riparazione del DNA e l'amministrazione di β -carotene

INTRODUCTION

As far as the health of human skin is concerned, ultraviolet radiation (UV) has been recognized as the single most damaging environmental factor. Its mechanisms of action have been well studied, and technologies have been developed to hinder these mechanisms. The protective role played by natural factors such as pigmentation and stratum corneum thickness has been investigated. Many of the physiological consequences of exposure to UV have been identified, and substances have been found, able to partially hinder the deleterious consequences of excessive exposure to solar radiation.

This paper summarizes some topics which may have not always received the attention they might deserve.

RESULTS

Photochemical considerations

With a molar extinction coefficient ranging around 15,000 at 260 nm, ribonucleotides and desoxyribonucleotides are among the substances with the highest UV absorbing power. This is even more relevant since above 290 nm, well in the UVB range, the molar extinction coefficient of ribonucleotides and desoxyribonucleotides is still of the order of 4,000, i.e. it is comparable to the molar extinction coefficients of several commercial sunscreens.

This is to say that DNA and RNA are the primary targets of UVB radiation, and it is well known that UV radiation damages DNA by generating cyclobutane-type pyrimidine dimers as well as 6-4 type pyrimidine dimers.

These dimers are mutagenic and, when not timely removed, they can cause specific mutations which can have undesirable consequences. These dimers are so widespread and have been

so widely studied, that they have taken all the "light" (if I dare write so) and the impression has been conveyed that they are the only relevant UV-induced damages.

Proteins have a much smaller molar extinction coefficient (of the order of 100) and direct damages caused by UV to proteins has been considered negligible when compared to the quantum yield of the generation of damages to DNA, even when taking into account that a cell contains about one hundred times more proteins than DNA. Indeed, damaged proteins are often ubiquitinated and removed, whereas non-repaired proteins trigger the expression of specific genes, such as the heat shock genes, but do not modify the genotype. Yet, one could surmise that in the time interval necessary to remove damaged proteins, the cells contains directly damaged proteins in so large amounts that they compare stoichiomerically, to the direct damages in the DNA, and this might not help the cell to feel very well.

Besides direct damages, though, which can only be avoided by the appropriate use of sunscreens, cells exposed to UV radiation experience also large amounts of indirect damages, which popular wisdom attributes to oxidative damages generated by radiation. Since UV is not a ionizing radiation, these secondary damages are the consequence of the absorption of UV by sensitizers, which then trigger a cascade of phenomena, which have been studied and described. For instance UVA is not absorbed by purified DNA. Yet, the complex formed by DNA with iron or copper, metals normally found in cells, has the capability to absorb UVA and hence (in the presence of Oxygen) to generate a perferryl radical which is able to cleave the phosphodiester backbone (Audic & Giacomoni, 1993).

Surprisingly enough, and notwithstanding widespread descriptions in the scientific literature, cosmetic scientists have devoted their attention

mostly to hydroxyl radicals and superoxide radicals, and have neglected the most important Reactive Oxygen Species (ROS), that is, Singlet Oxygen. This ROS is generated when a UV photon is absorbed by an aromatic heteronuclear molecule with a triplet state above a threshold value, hencefore enabled to transfer its energy to nearby Oxygen molecules and excite them into a state called "singlet".

Singlet Oxygen is responsible for mutagenic DNA damages such as 8-oxo-guanine, for protein carbonylation and for much of lipid peroxidation.

Singlet Oxygen is incredibly well quenched by β -carotene, with a rate constant of interaction, k , which is 100,000 times larger than the one for other lipids or DNA, and 1,000 times larger than the one for amino-acids in general (see below)

Rate constant of interaction of singlet Oxygen with biological molecules

- Protein damage
- (amino acids $k=30 \times 10^6$ l/mol.sec)
- Lipid peroxidation
- (β -carotene $k=13 \times 10^9$ l/mol.sec)
- (methyl linoleate $k=0,2 \times 10^6$ l/mol.sec)
- DNA damage
- ($k=0.5 \times 10^6$ l/mol.sec)

This is to say that iron-copper chelators and β -carotene do belong to the panoply of tools which could be used to fight the undesirable effects of solar exposure on human skin.

Photo-biological considerations

Within days after exposure of Caucasian or Asian skin to solar radiation, one observes a change in the pigmentation patterns and an increase of the thickness of the stratum cor-

neum. The protective role played by these two physiological parameters has often been discussed.

Results obtained by Bruls et al (1984) indicate that because of its thickness the stratum corneum filters off the impinging ultraviolet radiation and contributes somewhere between 20% and 70% of the protection offered by total epidermis (UV filters within the epidermis are mainly RNA molecules which constitute up to 1% of total epidermis). As far as melanin is concerned, experiments performed by Sheehan et al (1998) and by Sheehan et al (2002) with British volunteers indicate that a tan, induced by repetitive sub-erythral irradiations, affords only a modest protection against erythema and against epidermal DNA damage, corresponding to an SPF not larger than 2 or 3. The behavioral conclusion of these experiments was that a natural tan does not provide relevant protection against UV-induced damages, and that the use of sunscreens is recommended, when in the sun, even after having acquired a nice tan.

What is observed in British volunteers, though, can be different for other volunteers. Indeed a study by Tadokoro et al (2003) seems to indicate that the damage inflicted by UV radiation to epidermal DNA in the skin of volunteers from different ethnic groups might differ by as much as a factor of 10, and that the order of susceptibility to UV-induced DNA damage is as follows:

Caucasian > Asians > African American > Latinos

An interesting corollary in the series of experiments conducted by Tadokoro's team is that the relative permanence of DNA damage after exposure to UV (which gives a measure of the rate of DNA repair) differs in people from different ethnic origins, in the following order of increasing rate of repair

Caucasian < African American < Asians < Latinos

Photo-dermatological considerations

DNA is a relevant molecular target and DNA damage is a relevant parameter to learn about the action of UV radiation. The effects of UV on human skin range from erythema to aging, from pigmentation to immune-depression. Many of the physiological consequences of the exposure to UV radiation are mediated by DNA damage, in particular the so called immune-depression. It has been observed that upon exposure to ultraviolet radiation, the skin in rodents and in humans is no longer prone to contact hypersensitivity (CHS) or to delayed type hypersensitivity (DTH). Although Schleiijffers et al (2001) did show that the exposure UV does not affect the body's capability to produce antibodies after a normal vaccination, there is concern about the loss of capability to undergo CHS or DTH because CHS and DTH are mediated by antigen presenting cells, possibly following the same mechanisms which do allow dendritic cells in the epidermis to recognize and remove a transformed cell before it multiplies into a cancer. Vink et al (1997) showed that upon treating rodent skin with liposomes containing DNA repair enzymes, the activity of Langerhans cells is restored. In human healthy volunteers it has been shown by Fuller et al (1992) that oral administration of β -carotene (which is known to accumulate in the epidermis) has the advantageous consequences of protecting the epidermal immune system against the photo-induced depression of DTH.

CONCLUSIONS

UV-induced DNA damage mediates several undesirable physiological consequences, such as mutations and immune-depression, which are understood to be key steps in the onset of UV-

induced cancerogenesis.

Mechanisms for UVB- and UVA- induced DNA damages and mutations have been understood, specifically the direct absorption of UVB photons by DNA and the generation of perferryl radicals and of singlet Oxygen by UVB and UVA radiation. These two reactive species can be removed and quenched by the use of chelators and of β -carotene

Damages to DNA impair epidermal cell physiology, in particular the action of Langerhans cells which organize the watch against non-self intruders in the epidermis. Since the action spectrum of UV-induced immune depression is broad, it is recommended to associate broad spectrum UV absorbers with singlet Oxygen scavengers such as β -carotene and metal chelators and liposomes containing DNA-repair enzymes, in order to minimize the chances of accumulating DNA damage when exposed to solar radiation.

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