REVIEW

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UV light and oxidative damage of the skin

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1. Introduction

An excessive exposure of skin to ultraviolet light (UV) is thought to be directly associated with the initiation and promotion of skin tumours. Hence, the pathophysiological mechanisms of these processes are of high clinical interest. A possible cause of the increased incidence of skin tumors after a UV exposure seems to be the immunosuppressive action of radiation. As a result, the destruction of tumor cells by the immune system fails to appear. UV light results in a removal of the Langerhans cells in the epidermis and changes of their antigen presentation. In addition, UV light stimulates the secretion of different immunomodulatory cytokines [1]. UV-induced mutations based on DNA damages are additional explanations for the increased appearance of neoplasms. The effects of the UV light on the cellular level are extremely versatile. A part of the UV-induced cell alterations, such as the formation of pyrimidine dimers of the DNA, is directly caused by UV absorption. Besides, UV-light stimulates the release of different mediators like eicosanoids and reactive oxygen species (ROS) which are responsible for multiple pathophysiological effects [2–6].

2. UV-induced formation of ROS in the skin

The UV-induced formation of ROS in the skin is based on the absorption of photons of the radiation by cellular substances which are called photosensitizers or chromophores. Riboflavine, pophyrins, chinons, bilirubin, NADH, tryptophan, and melanin are examples of common photosensitizers in mammal cells [7, 8].

The energy of the photon transfers the photosensitizer in an excited state. This means, an electron is raised on an increased energy level and a singlet state is formed. The singlet excited photosensitizer can decay to the ground state with release of energy in the form of fluorescence or heat. Alternatively, the photosensitizer undergoes intersystem crossing (ISC) into a triplet state. The triplet state is characterised by two electrons with parallel spin and a relative stability. This state leads to secondary reactions which include two different mechanisms. The so-called type I mechanism is characterised by the reaction of the excited photosensitizer with a substratum which becomes a radical itself. In the type II mechanism, the photosensitizer reacts with oxygen which is converted into a singlet oxygen and a superoxide anion. Because of their high reactivity, the radicals formed by the type I and the type II mechanism are unstable and initiate further reactions. The spontaneous dismutation of superoxide anion leads to hydrogen peroxide (H_2O_2). The enzyme superoxide dismutase (SOD) enormously accelerates this reaction (eq. 1).

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (1)$$

By a further transfer of an electron, the high reactive hydroxyl radical is formed. This occurs via the Fenton reaction (eq. 2) or, alternatively, via the Haber-Weiss reaction in which the superoxide anion serves as an electron donor (eq. 3).

$$H_2O_2 + Fe^{2+} + H^+ \rightarrow {}^{\bullet}OH + Fe^{3+} + H_2O \qquad (2)$$

$$H_2O_2 + {}^{\bullet}O_2^- + H^+ \rightarrow {}^{\bullet}OH + O_2 + H_2O \qquad (3)$$

The ROS formed by the transformation reactions are different with regard to their reactivity. The most reactive species is the hydroxyl radical which reacts immediately at the place of its generation with all biological material [11].

3. Biological effects of ROS

The most important cellular targets of ROS are lipids, DNA, proteins, and carbohydrates (Fig.). The phospholipids of membranes consisting of polyunsaturated fatty acids are especially endangered due to the ROS-induced lipid peroxidation. Possible consequences of the lipid peroxidation are changes in the permeability of membranes and activation of phospholipases. As a consequence of their reactivity, hydroxyl radicals and singlet oxygen are the main initiators of the lipid peroxidation. The degradation products of the lipid peroxidation malondialdehyde and 4-hydroxynonenal are cytotoxic, mediate DNA damages, and are chemotactic for immunocompetent cells [12, 13].

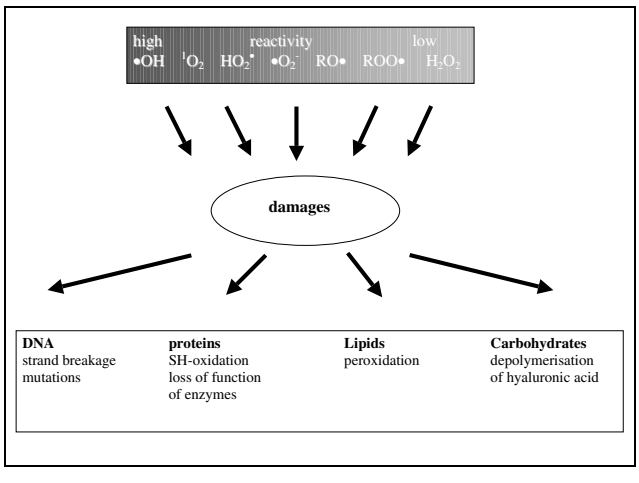

Fig.

Free radicals act as mutagen, too. DNA damages are caused by the attack of hydroxyl radicals to guanine. An important oxidation product is 8-hydroxyguanine, which mispairs with adenine resulting in G·C to T·A transversion mutations. Thus, 8-hydroxyguanine is considered a premutagen lesion in mammal cells [14, 15]. Singlet oxygen has been shown to induce DNA strand breakage and DNA protein cross-linking [16, 17]. Moreover, proteins can be denatured by oxygen species. Owing to oxidation of sulphur-containing amino acids such as cysteine and methionine, a loss of function of enzymes may occur. Pepsin, ribonuclease, and calmodulin are examples for ROS-induced inactivation of enzymes. The oxidative conversion of glutathione in glutathione disulfide results in the inhibition (pyruvat kinase, adenylat cyclase) as well as the activation of enzymes (collagenase, glucose-6-phosphate dehydrogenase) [9].

The depolymerisation of hyaluronic acid is an example for a radical-induced damage of carbohydrates [18]. Moreover, the cleavage of desoxyribose by hydroxyl radicals to malondialdehyde is described. This reaction is of use for detecting hydroxyl radicals [19].

The formation of skin tumors and the premature skin aging are clinical consequences of a UV-elevated oxidative stress. Matrix-metalloproteinases (MMPs) seem to play a significant role in either process. The activity of these proteolytic enzymes leads to the destruction of the connective tissue and thereby promotes the invasion of tumors and the formation of metastases as well as photoaging.

ROS may act in these pathophysiological processes as signal mediators. They cause an activation of the redox-sensitive transciption factor AP-1, which stimulates the transcription of MMPs. The activity of the MMPs is controlled by specific inhibitors, the so-called tissue inhibitors of metalloproteinases (TIMP). Interestingly, it could be shown that ROS are able to inhibit TIMP and thereby have a twofold negative influence on these processes [20, 21].

An additional possible mechanism for the radical-induced damage of the skin has been shown by Clement-Lacroix et al. [22]. It is well-known that the decrease in the number of Langerhans cells in the epidermis or changes in their antigen presentation represent early events in the formation of skin tumors. The authors demonstrated that the UVA-induced damage of the Langerhans cells in the human skin is associated with an elevated oxidative stress. Moreover, vitamin E has been shown to partially inhibit these processes.

4. Nitric oxide

Nitric oxide (NO) is a biological mediator implicated in a variety of biological processes of nearly every organ system. The biosynthesis of NO is based on the cleavage of the amino acid L-arginine by means of NO synthases (NOS) to NO and citrulline. The NOS can be subdivided into at least three isoforms: The constitutively expressed neuronal (n-NOS, NOS I) and endothelial forms (e-NOS, NOS III) as well as the inducible isoform (i-NOS, NOS II). All types of dermal cells are able to express the different isoforms and to release NO [23]. UV-light is described as a possible stimulus for the NOS of the skin. Up to now, the exact participation of the different isoforms in this NO release is still unidentified. There are some hints suggesting the involvement of the constitutively expressed NOS in the UV-induced NO release of the skin. However, an increase in the expression of the i-NOS after UV-light has also been described [24–26]. The importance of the dermal NO release has frequently been discussed for the last years. Several studies investigated the influence of NO on the proliferation and differentiation of keratinocytes. In this connection, a report of Krischel et al. [27] showed that a treatment of keratinocytes with low doses of a NO donor increased proliferation whereas high doses had cytostatic effects and resulted in an increase in differentiation. Similar results have been published by Vallette et al. [28]. The authors attributed the antiproliferative effects directly to NO and interpreted the induction of the terminal differentiation as an effect of peroxynitrite ($ONNO^-$). The latter is formed by the reaction of superoxide anion (O_2^-) with NO. The findings of a recently published report, however, suggest that NO donors inhibit the processes of the terminal differentiation in keratinocytes [29].

It is a controversially discussed question whether the UV-induced cutanous NO release is a part of the radiation-induced oxidative stress or, alternatively, is a component of the endogenous antioxidative defence system. In agreement with the latter, Romero-Graillet et al. [30] demonstrated that NO produced by UV-irradiated keratinocytes stimulates the melanogenesis of melanocytes.

Suschek et al. [31] demonstrated the protection of endothelial cells from UVA-induced apoptosis by NO. For this, the authors stimulated the endogenous NO release using a cytokine mix. Moreover, exogenous administrated NO protected the cells from apoptosis as well. A recently published paper from the same group seems to confirm the photoprotective action of NO. In that study, the NO donor S-nitro-cysteine protected endothelial cells from UVA-induced cell death. In addition, a reduction of the lipid peroxidation was observed [32].

In contrast to these findings, there is evidence that UV-induced NO contributes to the radiation mediated skin damage. In this context, a participation of NO in the UV induced erythema is discussed. Topical NOS inhibitors prevented a UVB-induced erythema in human volunteers [33]. In keratinocytes a treatment with L-arginin caused an increased sensitivity against UVA-induced cell damage [34]. Recently, we could demonstrate that the NO radical scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) and the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) protect human keratinocytes from UVA-induced damage, whereas the essential cofactor of NOS tetrahydrobiopterine (BH4) increased a radiation-mediated damage [35].

The enumeration documents the considerable interest in the role of the UV-induced NO release by dermal cells. Beyond this, it becomes obvious that the meaning of these processes for the physiology of the skin is only partly known.

5. Antioxidative defence system of the skin

5.1. Enzymes

As an organ permanently exposed to the environment, the skin has developed a complex system in order to protect itself from oxidative stress. This defence system consists of enzymes and non-enzymatic radical scavengers. A component of the first group is the SOD which catalyses the dismutation of superoxide anions to hydrogen peroxide. This enzyme can be subdivided in a cytosolic, copper- and zinc-containing (Cu, Zn-SOD) and in a mitochondrial, manganese-containing (Mn-SOD) [36, 37] isoform. The glutathione peroxidase (GSH-Px) and the catalase (CAT),

which catalyse the further detoxification of hydrogen peroxide, are other parts of the enzyme-based defence system against oxidative stress [38]. The distribution profile of these enzymes in the skin corresponds with the extent of oxidative stress. Their concentration as well as their activity is accordingly higher in the epidermis than is in the dermis [39]. Sasaki et al. investigated the UV protective potential of SOD in cultivated human keratinocytes. The authors demonstrated that an inhibition of the SOD leads to an increase in the UV-induced lipid peroxidation and to an enhanced cell death [40]. Petersen et al. showed that the inhibition of the CAT and the GSH-Px amplifies a UV-induced DNA damage [41]. Conversely, it has been shown that a stimulation of the GSH-Px increases the resistance of cultivated fibroblasts, keratinocytes, melanocytes, and human skin against irradiation-induced oxidative stress [42–44]. The findings of Guarrera et al. suggest that the polymorphic light eruption (PLE) is a clinical manifestation of a reduced content of antioxidative enzymes in the epidermis. The authors demonstrated a deficit of CAT in the epidermis of patients with PLE in comparison with the skin of healthy volunteers [45].

5.2. Radical scavenger

Non-enzymatical radical scavengers can be subdivided based on their solubility. Vitamin C and glutathion represent the most important hydrophilic radical scavengers. The most significant lipophilic radical protector is vitamin E [46]. Its outstanding role has been documented by a large number of studies. Igarashi et al. showed that a vitamin E deficit diet caused a considerable increase in the lipid peroxidation rates as compared to control animals fed with standard diet [47]. Thiele et al demonstrated that an irradiation of the human stratum corneum with a suberythema dose of solar simulated UV-light results in a remarkable depletion of vitamin E [48]. Different authors investigated whether an additional supplementation of the skin with vitamin E protects the skin from radiation-induced damage. Several groups demonstrated that topical vitamin E diminished the formation of an UVB-induced erythema [49–51]. In addition, there is good evidence for an photoprotective effect of oral administered vitamin E as well [52, 53].

Interestingly, vitamin E is not only a radical scavenger but also a pharmacologically active compound. These pharmacological actions of vitamin E might contribute to its photoprotective potential [54–57].

5.3. Gene induction

The work of Vile et al. showed an alternative, endogenous, antioxidative mechanism. The authors observed that fibroblasts, pre-irradiated with UVA, are considerably more resistant against a subsequent exposure to UVA than are cells that were treated for the first time with UVA irradiation. The induction of the enzyme heme oxygenase might be the explanation for this adaptive response to oxidative stress. The authors suggest an indirect protective role for the heme oxygenase based on its capacity to induce the expression of the iron storage protein ferritin [58, 59]. Interestingly, it was shown that the basal ferritin level in cultivated keratinocytes is 3–7-fold higher than in fibroblasts [60], an observation which could be based on the various extent of oxidative stress in the different skin layers. The epidermal keratinocyte is much more exposed to UV-radiation than is the dermal fibroblast.

Table: Formation of reactive oxygen species in the skin

A)	$^{0}\text{photosensitizer} + h^{\bullet}v \rightarrow {}^{1}\text{photosensitizer} \rightarrow {}^{3}\text{photosensitizer}$ (stable triplet-state)
B)	*type I-mechanism* $^{3}\text{Photosensibilisator} + \text{substrate} \rightarrow \text{substrate radicals} \rightarrow \text{secondary reactions}$
B)	*type II-mechanism* $^{3}\text{photosensitizer} + {}^{3}O_2\ (\text{triplet oxygen}) \rightarrow {}^{1}O_2$ (singlet oxygen) $+ O_2$ (superoxide anion)

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