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UV-generated free radicals (FR) in skin: Their prevention by sunscreens and their induction by self-tanning agents

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Abstract

In the past few years, the cellular effects of ultraviolet (UV) irradiation induced in skin have become increasingly recognized. Indeed, it is now well known that UV irradiation induces structural and cellular changes in all the compartments of skin tissue. The generation of reactive oxygen species (ROS) is the first and immediate consequence of UV exposure and therefore the quantitative determination of free radical reactions in the skin during UV radiation is of primary importance for the understanding of dermatological photodamage. The RSF method (radical sun protection factor) herein presented, based on electron spin resonance spectroscopy (ESR), enables the measurement of free radical reactions in skin biopsies directly during UV radiation. The amount of free radicals varies with UV doses and can be standardized by varying UV irradiance or exposure time. The RSF method allows the determination of the protective effect of UV filters and sunscreens as well as the radical induction capacity of self-tanning agents as dihydroxyacetone (DHA). The reaction of the reducing sugars used in self-tanning products and amino acids in the skin layer (Maillard reaction) leads to the formation of Amadori products that generate free radicals during UV irradiation. Using the RSF method three different self-tanning agents were analyzed and it was found, that in DHA-treated skin more than 180% additional radicals were generated during sun exposure with respect to untreated skin. For this reason the exposure duration in the sun must be shortened when self-tanners are used and photoaging processes are accelerated.

Keywords: Skin; UV irradiation; Free radicals; Self-tanner; Maillard reaction; ESR spectroscopy

1. Introduction

The effects of chronic sun exposure on skin are apparent when skin not typically exposed to the sun and skin regularly exposed to the sun are compared. While the sun is not the only aetiological factor in the dynamic process of skin aging, it is the primary exogenous cause among several internal and environmental elements. Thus, photoaging is a subset of extrinsic skin ageing.

Solar UVB (280–320 nm) and particularly UVA (320–400 nm) radiations have a capacity to generate reactive chemical species, including free radicals, in cells. These intermediates have been shown to be involved in various biological effects in skin (e.g. erythema, skin aging, skin wrinkling, cancer).

The molecular oxygen (O_2) present within skin cells in the mid-lower levels of the epidermis is a primary target for UV light waves that penetrate the skin.

The reactive oxygen products and other biologically important free radical species are usually very unstable in biological material due to their high reactivity. Free radicals have a characteristic half-life due to their chemical reactivity. Some radicals are stable enough to diffuse across biological membranes; others are so reactive, that they react in the chemical microenvironment at their site of formation. Very reactive free radicals (hydroxyl radicals) cause biological damage only if generated in close proximity to a potential target molecule (e.g. DNA), because they are immediately scavenged by the high concentration of organic molecules in the cell. If they are to cause cell damage directly, they need to be generated directly at the critical cellular target site [1].

Free radicals of intermediate reactivity are able to diffuse over significant distances and may then react with some specificity

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and selectivity with target molecules. They are the most likely species to lead to direct biological damage. Persistent free radicals are rather biologically unimportant with respect to direct cell damage. Persistent free radicals of physiological relevance are melanin radicals, which can be detected directly in skin and hair.

Numerous methods are used to quantify the skin damage induced by UV exposure. The most common in cosmetic industry is the SPF (sun protection factor) method that measures the MED (middle erythema dose), thus the time necessary to develop an erythema. Since skin erythema is caused mainly by the UVB radiation, due to its short wavelength and its superficial penetration depth into the epidermis, this SPF method quantifies only the biological UVB damage. Other methods quantify biological endpoints as skin pigmentation (PPD method, permanent pigment darkening) or DNA-fragmentation. All of these endpoints are very advanced steps in a biological reaction cascade in the skin, that has its beginning with the generation of free radicals. The accurate measurement of these UV-induced free radical species directly in the exposed skin is on the basis of a method able to characterize the benefits or risks of cosmetical and/or pharmaceutical dermatological products. The quantification of free radicals and free radical reactions in the skin could be a valid method for the estimation of UV damage. The quantification of free radical reactions is realized by electron spin resonance (ESR) spectroscopy. Herein we present a method for ex vivo examination of skin biopsy samples, which enables an analysis of free radical reactions as a function of UV irradiation dose, and UV intensity. From the relationship between applied UV dose and radical concentration in a skin sample a calibration curve is constructed, that allows to analyze, if a substance applied on the skin protects against UV-induced free radicals or even enhances UV damage. Sunscreen agents protect against UV-induced free radicals, if they contain UV filters that adsorb or scatter the UV radiation mainly in the UVA range (280–400 nm) and if they are sufficiently photostable to ensure a protection over longer radiation times (UV doses). Some examples of the protective effect of chemical and physical UV filters are discussed here. On the other hand there are chemicals that can enhance the free radical reactions in skin. Herein we present a study on processes of the radical induction by self-tanners as DHA (dihydroxyacetone) and erythrulose. These sugar compounds are commonly used in cosmetic self-tanning products to obtain a sunless coloring of the skin. The chemical reaction process of these sugar compounds is the Maillard reaction, where the sugars react with the proteins of the keratinocytes in the first layers of the stratum corneum and epidermis. During UV radiation free radicals and mainly superoxide anion ($O_2^{\bullet -}$) are produced, that can react with the ketoamines (Amadori products) and other intermediates of the Maillard reaction. This leads to oxidation of the sugar derivates and the consequent radical chain reactions cause a dramatic increase in the radical injury of the skin.

Both the protective effect of sunscreens as well as the radical induction effects of self-tanners can be quantitatively determined by means of the same RSF (radical sun protection factor) method [2].

2. Experimental

2.1. Materials

2.1.1. Skin biopsy samples

Skin biopsy samples from pig were used in all experiments. Pig skin has the greatest similarity to human skin and has the main advantage of a high structural and functional homogeneity. The ears of 6-months-old pigs from local slaughter were washed, the cartilage and the subdermal fat was removed, the skin was cut into 1 cm × 1 cm pieces and stored in PBS buffer until used at 4 °C for maximum 6 h.

2.1.2. Marker for ESR analysis

PCA probe—2,2,5,5-tetramethyl piperidine-*N*-oxyl (Sigma–Aldrich, Germany) at 1 mM concentration in water was used to detect free radical reactions.

2.1.3. UV filters

Commercially available sunscreen products containing different concentrations of four chemical UV filters (B1, A, B2, AB) were used.

2.1.4. Self-tanning agents

DHA (dihydroxyacetone) was purchased from Sigma–Aldrich, Germany, erythrulose from Kraeber, Germany and liposomal encapsulated DHA from ROVI Cosmetics International, Germany. Five percent, 10%, and 20% (w/w) concentrations of all tanning agents were prepared in distilled water and used for the experiments within 12 h.

2.2. Methods

Direct evidence of free radical formation in skin tissues following exposure to UV radiation can be obtained by ESR spectroscopy at low temperature (77 K). However, these signals are very broad and usually give only limited information about the chemical identity of the free radical structure. For quantitative determination of radical reactions as a function of UV dose neither this direct detection nor spin trapping agents are suitable. There are multiple requirements to an ESR probe able to monitor radical reactions in skin at room temperature: the probe must penetrate into the dermal and epidermal layer of the skin without signal intensity loss to the enzymatic and non-enzymatic biological reaction. Further, it must be photostable and non-degraded by UV radiation. It must react with the ROS and free radicals (FR) generated inside the skin during UV radiation. The nitroxyl spin probe PCA fulfils all these requirements and is a suitable probe for the detection of radical reactions by ESR spectroscopy [3–5].

2.2.1. UV irradiation

A sun simulator SOL F2 (Hönle AG, Germany) with UVA 25 mW/cm^2 and UVB 2.6 mW/cm^2 was used for all experiments.