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The Fatal Effect of Self-Tanning Agents during UV Irradiation

Keywords: skin, self tanning agent, UV irradiation, maillard-reaction, free radicals

Introduction

Dihydroxyacetone (DHA), a three-carbon sugar, is the main browning ingredient in commercial sunless tanning formulations. DHA preparations have been used for many years and are currently highly popular for producing temporary pigmentation resembling an ultraviolet (UV) induced tan. 10% of the US population currently tan indoor (1,2). Pigmentation develops over a period of hours after application of DHA and remains for several days. Similar to melanin, DHA-induced pigmentation absorbs light throughout the visible spectrum. DHA-induced pigment is much less photo protective than melanin against UV radiation, however, because it has relatively lower absorption in the UV wavelength range. DHA pigment is only moderately protective against UVA radiation showing protection factors of approximately 2 – 5 (3,4). Recently, multiple applications of DHA have been shown to induce pigmentation that is sufficient to protect uninvolved skin during psoralen plus UVA (PUVA) therapy for psoriasis (5). This approach allowed higher UVA doses to be used and resulting in fewer treatments than standard PUVA regime.

DHA-induced pigmentation forms in the stratum corneum, rather than in deeper epidermal layers. The chemistry leading to DHA pigments is similar to that established for reactions of other sugars with compounds containing amino groups and has been termed Maillard reactions. This series of reactions has been extensively studied because it is largely responsible for the non-enzymatic browning of sugar-containing foods (6). The pigments formed, often called melanoidins, are complex collection of high molecular weight, visible-absorbing chromophores that are produced through a series of non-enzymatic chemical steps.

Abstract

Using the recently developed RSF (Radical Skin Protection Factor) method, the radical induction capacity of self tanning agents as dihydroxyacetone (DHA) was investigated. The reaction of the reducing sugars used in self tanning products with amino acids in the skin (Maillard reaction) leads to the formation of Amadori-products that generate free radicals during UV irradiation. Three different self tanning agents were analysed and it was found that particulary DHA increased the amount of generated free radicals up to 180% during sun exposure. For this reason the exposure duration in the sun is decreased by using self tanning agents. Photo aging processes in the skin are accelerated at the same time.

Free radicals, called melanoidin radicals, are the end result of the Maillard reaction (7). They are formed during the Maillard reaction between carbohydrates, DHA, and amino groups in the skin proteins, such as from lysine and arginine, leading to cation radical intermediates. In contrast to the short-lived UV-induced oxygen free radicals like hydroxyl and super oxide anion radicals the melanoidin radicals are highly stable.

The Maillard browning reaction between carbohydrates and amines is part of an extensive series of reactions that is the basis for the brown color caused by the »sunless tanning« agent dihydroxyacetone in self-tanning products. The initial stages of the reaction are quite complex, but the ultimate products are brown polymers known collectively as melanoidins.The first steps of the reaction of these sugars with the amino acids of the cell components of the stratum corneum and the epidermis lead to ketoamines called Amadori products. The enolization processes of these Amadori products and their oxidation products are known to produce free radicals. Particularly, numerous fructose-amino acids (Amadori-compounds) contribute to the formation of oxygen free radicals and their subsequent oxidative damage to proteins (8). The formation of cross-linked AGE-proteins (Advanced Glycation Endproducts) is a consequence of Maillard reaction in skin **(Fig. 1)**. There are also evidences that Amadori products trigger oxidative modification of lipids via the generation of superoxide, and implied the involvement of lipid glycation along with membrane lipid per-

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oxidation in the pathogenesis of diabetes and aging (9).
In order to show that radicals in the skin are produced by the Maillard reaction, electron spin resonance studies were performed *in vitro* and *in vivo* (7,10).
The investigation of the effects of DHA on cell survival and proliferation of a human keratinocyte cell line showed dose- and time dependent morphological changes, chromatin condensation, cytoplasmic budding and cell detachment. Several dead cells were observed after long-time (24 h) incubation with 25 mM DHA or more (11). Furthermore, an extensive decline in proliferation was observed 1 day after DHA exposure for 24 h. Preincubation with antioxidants prevented the formation of DNA strand breaks. The DHA toxicity may be caused by direct redox reactions, with formation of free radicals/ROS as the crucial intermediates. The genotoxic capacity of DHA raises a question about the long-term clinical consequences of treatment of the skin with this commonly used compound.
Other self tanning agents used in combination with DHA are erythrulose, glyceroaldehyd, or hydroxymethylglyoxal all have in common the characteristics of reducing sugars.
These later insights including the contradictions between beneficial and harmful effects of self tanning agents have challenged the measurement of the RSF (12) of cosmetic formulations containing self tanning agents like DHA or erythrulose applied on skin and followed by UV irradiation.

■ Materials and Methods

Skin biopsies from pig were used in all experiments. Pig skin has the greatest similarities to human skin and has the main advantage of a high structural and functional homogeneity. The ears of 6 month old pigs from local slaughtery were washed, the cartilage and the subdermal fat was removed, the skin was cutted into 1 x 1 cm pieces and stored on PBS buffer until used at 4 °C.
Free radical indicator: Oxygen and carbon centered free radicals generated in skin during UV irradiation were detected by using radical traps (13) on the basis of nitroxyl compounds.

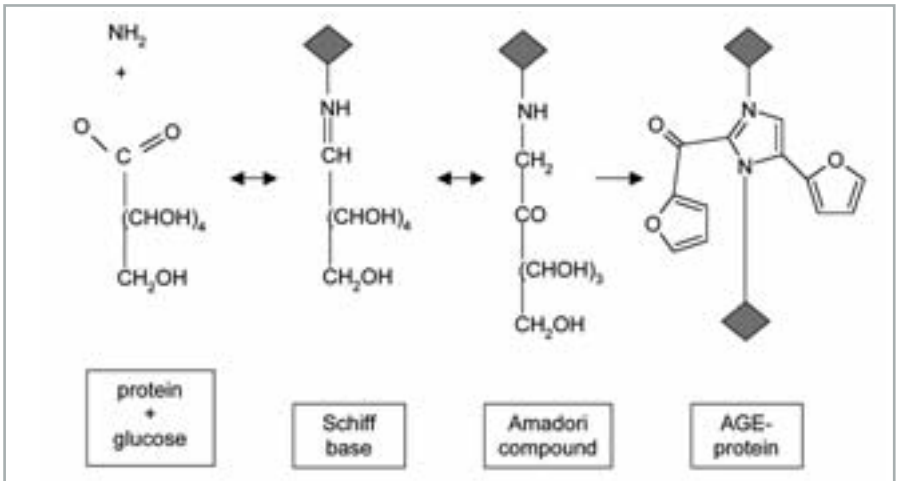

Fig. 1 Schematic representation of the Maillard reaction

Self tanning agents: DHA (dihydroxyacetone) was purchased from Sigma-Aldrich, Germany, Erythrulose from Kraeber, Germany and liposomal encapsulated DHA from ROVI Cosmetics International, Germany. 5%, 10%, and 20% (w/w) concentrations of all tanning agents were prepared in distilled water and used for the experiments within 12 h.

UV irradiation: A sun simulator SOL F2 (Hönle AG, Germany) with E(UVA) = 25 mW/ cm² and E(UVB) = 2,6 mW/cm² was used for all experiments.

Instrumentation for radical detection: A X-band ESR spectrometer Miniscope MS200 Magnettech, Germany was used for *ex vivo* radical detection. A special tissue cell for skin measurements was used.

Experimental process: 1x1 cm² skin biopsies were placed on a paper imbued with a 1 mM solution of the radical indicator. The test products were applied on the epidermal side of the skin biopsies and allowed to penetrate for different times. Afterwards, skin biopsies of 4 mm diameter were taken with a biopsy punch, the skin was fixed on the tissue cell and the first ESR spectrum was recorded. The skin in the tissue cell was UV irradiated with different UV doses (corresponding to different irradiation times). After that process the ESR spectrum of the skin biopsy was measured and the data were analyzed.

■ Results and Discussion

RSF method

For the characterisation of the free radical protection or generating effect of sunless tanning formulations applied on the skin the Radical Skin protection Factor (RSF) was measured.
The RSF presents the ratio between the number N of generated free radicals in the untreated (unprotected) and treated (protected) skin assuming the same applied UV dose (constant irradiance, variable irradiation time) for both or the variation of the time staying in the sun after topical application of the product assuming the generation of the same amount N of free radicals like for the untreated skin.

$$RSF = \frac{N(\text{free radicals})_{untreated}}{N(\text{free radicals})_{treated}}$$

The amount of free radicals in the skin induced by UV radiation is determined by the reaction of the short lived free radicals with the semistable nitroxyl probe. The signal intensity of the nitroxyl probe was detected by ESR spectroscopy as a function of UV doses (constant irradiance, variable exposure time). The RSF can quantitatively analyse the protection effect **(Fig. 2)** of sun protection products such as sunscreens containing chemical or physical UV filters. A RSF > 1 indicates that an applied formulation provides an UV protection (radical protector) in terms of prevented free radicals. On the other hand a RSF factor < 1

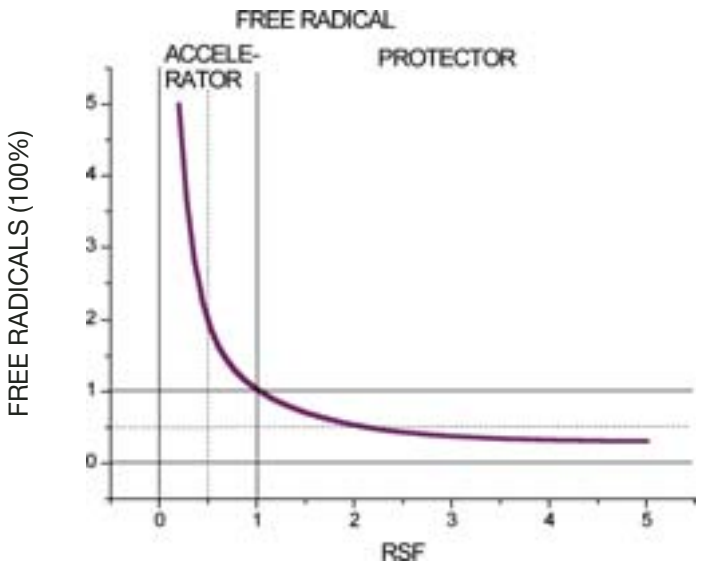

Fig. 2 The RSF as a function of topical applied substances responsible for free radical induction or protection in the skin

indicates that a formulation applied on the skin enhances the radical injury (radical accelerator) inside the skin caused by UV radiation. Normal untreated skin results in a RSF = 1.

UV protection of skin treated with self tanning agents

During the Maillard reaction occurring in DHA treated skin ketoamine intermediate products are formed, wich are susceptible to UV irradiation leading to a dramatic increase in free radical generation. In order to quantify this radical formation the RSF method has been applied for three self tanning ingredients: dihydroxyacetone solved in water; dihydroxyacetone encapsulated in liposomes; and erythrulose dissolved in water. Both the applied concentration and the penetration time of the tanning formulations

concentration	RSF								
	penetration time, min								
	DHA			liposomal DHA			erythrulose		
	10	20	40	10	20	40	10	20	40
5 %	0,93	0,95	1,00	1,00	0,98	0,86	0,98	0,83	0,72
10 %	0,81	0,74	0,64	0,92	0,82	0,76	0,97	0,81	0,72
20 %	0,79	0,67	0,55	0,75	0,71	0,75	0,65	0,73	0,69

Table 1 RSF values for DHA-, erythrulose-, and liposomal DHA-treated skin

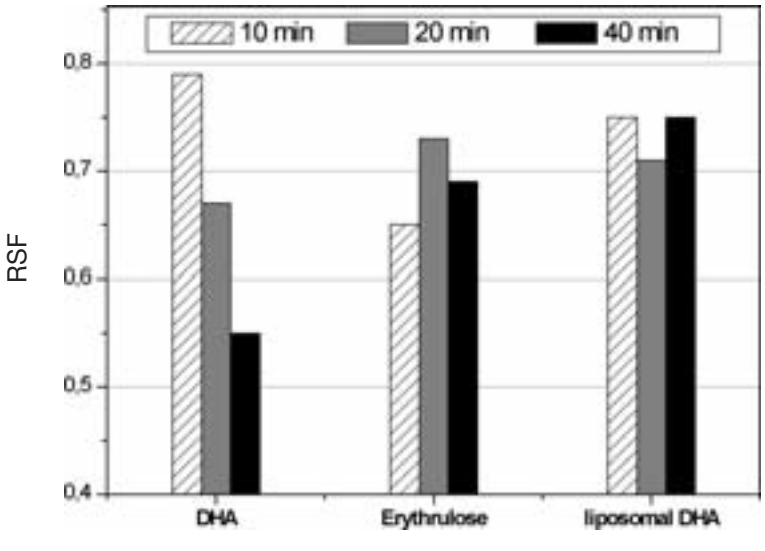

Fig. 3 RSF values of skin treated with 20 % (w/w) water solution of DHA, erythrulose, and liposomal encapsulated DHA (ROVISOME DHA

before UV radiation have been changed for all three products. **Table 1** and **Fig. 3** show the results of the RSF investigation. It is worth noting that the coloring effect of all products appears about 12 hours after the application of the tanning agent, but the Maillard reaction begins to take place within the very first minutes. DHA and the liposomal DHA results in a orange-brown color; erythrulose gives a lighter yellowish color. As shown in the results, the RSF of all products is arranged for RSF < 1. That means that self-tanning agents promote the generation of free radicals and enhance the free radical injury during UV radiation compared to untreated skin. As a tendency, the higher is the applied concentration of tanning agents, the more free radicals are generated. The highest effect was observed for a pure DHA so-

lution. The liposomal encapsulation of DHA increases moderately the number of UV generated free radicals. This effect is caused by different penetration kinetics of the liposomes into the epidermal layers of the skin and by lower reaction capacity of the sugars with peptides and amino acids due to the liposomal encapsulation. At higher concentrations also erythrulose enhances the radical formation, although its coloring efficacy is well below opposite to DHA.

For the analysis of the importance of the results presented in **Table 1** and **Fig. 2** the following consideration is useful. A RSF = 0.5 means that the radical protection of the skin is halved by the applied product under UV irradiation. It means also a reduction of the individual exposure time at 50%.

On the other hand, a factor of 0.5 means that inside the skin a free radical injury was measured that would have occurred if the skin was irradiated with twice the UV dosis of the control.

In **Table 2** the increase (percent) of UV generated free radicals is listed for the three tanning products as a function of different concentrations and penetration times.

After an application time of 40 minutes of a solution of 20% DHA on the skin, the number of UV generated free radicals was increased over 180% compared with the untreated skin (100%).

	additional free radicals in skin, %		
penetration time	DHA	Liposomal DHA	Erythrulose
10 min	127	133	154
20 min	149	141	137
40 min	182	133	145

Table 2 Additional free radicals produced in skin treated with 20% of self tanning agents compared to untreated skin

■ Conclusion

The quantitative determination of free radicals in skin during UV irradiation is an important requirement when the biological consequences of sun exposure are under study. Particularly, skin aging, wrinkling, dermatosis and other pathological conditions are strongly thought to be primarily caused by UV induced free radicals in the epidermal and dermal layer of the skin. Electron spin resonance (ESR) spectroscopy is a valid tool for the determination of free radical reactions and the herein presented RSF method (Radical Skin Protection Factor) is a standardized and validated tool for the quantification of radical injury in skin biopsies. By this method both the protection effect of sunscreens (UV filters) and the radical induction of self tanning agents can be investigated and quantitatively determined. The efficacy of sunscreen formulations in protecting the skin against UV induced free radicals depends on the composition of UV filters. Mainly UVA filters that absorb or scatter the radiation in the UVA region (280-400 nm) contribute largely to high RSF values. The higher is the RSF, the longer is the resting time in the sun. The radical induction capacity of self tanning agents was evaluated using the same method. Different tanning agents that undergo Maillard reaction in the skin were tested in different concentrations and for different penetration times. During UV radiation of the treated skin the first reaction products and intermediates of the Maillard reaction are susceptible to UV and generate a huge amount of additional free radicals inside the skin. After 40 minutes treatment with 20% DHA solution an RSF factor of 0.55 was found. That means that more than 80% of additional free radicals are generated in the skin during UV exposure. Contrary to untreated skin the number of UV generated free radicals was changed from 100% to 180%.

Paradoxically, also skin-lightening agents as kojic acid seems to induce the generation of free radicals during sun exposure. The activity of antioxidants in neutralizing the free radicals induced by UV irradiation of skin treated with self tanning agents or skin lighteners is currently under study.

The following facts can be seen as important results of the performed study:

- DHA decreases considerably the resting time in the sun characterized by values of RSF < 1.
  (For a formulation with 20% DHA resulting in a RSF = 0.55 the exposure time of a subject is decreased by about 50% of his individual resting time in the sun).
- The detrimental effect of DHA during sun exposure can be reduced by the encapsulation of DHA in liposomes.
- A combination of a self tanning agent with an antioxidant in a cosmetic formulation enhances the antioxidative Power (AOP) of the skin and can minimize the additional detrimental effects caused by UV irradiation.
- The prevention of early photo aging processes in the skin requires a minimization of the UV irradiation time of subjects treated with self-tanning agents.

In order to minimize the fatal effects of self-tanning cosmetics, the addition of radical scavenging mechanisms seems to be indispensable. Suitable protective strategies could be the addition of UV-filters on one side and the addition of antioxidants on the other side. Both strategies are currently under study and the results are to be published in a forthcoming part II.

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