Transfer of ultraviolet photon energy into fluorescent light in the visible path represents a new and efficient protection mechanism of sunscreens

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Abstract. The development of sunscreens with high sun protection factor (SPF) values but low filter concentrations is the ultimate goal. The purpose of the present study was to investigate why a sunscreen spray and cream with different concentrations of the same UV-filters provided the same SPF. Therefore, the homogeneity of the distribution of both sunscreens was investigated by laser scanning microscopy (LSM) and tape stripping (TS). Additionally, the energy transfer mechanisms of the sunscreens on the skin were analyzed. The TS and LSM showed a better homogeneity of the distribution of the spray. With Wood’s light, a total absorption of the irradiation was detected in the spray area. In contrast, after cream treatment, an intensive fluorescent signal was observed. It was demonstrated that this fluorescent signal was caused by nonthermal energy transferred from the UV-filters to one compound of the cream releasing its excitation energy by fluorescence. This nonthermal energy transfer seemed to be the reason for the high efficiency of the cream, which is subjected to thermal relaxation. The transfer of UV photon energy into fluorescent light represents a new approach to increase the efficiency of sunscreens and could form the basis for a new generation of sunscreens.

Keywords: sunscreens; sun protection factor; universal sun protection factor; homogeneity; laser scanning microscopy; tape stripping.

1 Introduction

Whereas UVB radiation is fully absorbed by the stratum corneum and the upper layers of the epidermis, up to 50% of incident UVA radiation enters Caucasian skin and penetrates deep into the dermis.

Exposure to UV radiation with its ionizing properties is the origin for a number of adverse events, which are partly caused by cracked hydrogen compounds in the biomolecules, ranging from sunburn to skin aging and induction of cutaneous malignancies.

Photoprotection has become very popular in recent decades and is involved in a public policy concern. The protective measures that can be taken are avoidance of the sun, protection through clothing, and the use of sunscreen filters. The latter are shown to have a protective role against photocarcinogenesis, photoimmunosuppression, and photaging, and have become an essential armament for dermatologists in providing protection to human skin against adverse effects of solar radiation.

In a first step, sunscreens are effective for prevention of erythema, the endpoint used in sun protection factor (SPF) determinations. The SPF is defined as the ratio of the dose of UVR (290 to 400 nm) required to produce 1 minimal erythema dose (MED) on sunscreen-protected skin (after application of 2 mg/cm² of product) over the dose to produce 1 MED on unprotected skin.

However, the SPF contains no information about UVA, which also causes damage to the skin including skin cancer.

Additionally, the universal sun protection factor represents a well described ex vivo method, which combines in vitro tape stripping (TS) with in vivo spectroscopic measurements. In this case, the real UV filter distribution determined by the skin structure is transferred undisturbed to the tape strips, which are analyzed spectroscopically. This value describes the protection properties of sunscreens, not only in the entire UV but also in the visible and infrared (IR) spectral ranges. This is of particular importance, as recently it could be demonstrated that visible and IR irradiation of the sun can also cause the formation of free radicals and skin damage.

Consequently, the protection efficacy of a sunscreen product is determined by the absorption properties of the UV-filter substances, by the homogeneity of the distribution of the sunscreen on the skin, and by the annihilation of the absorbed photon-energy in the skin.

Therefore, the creation of sunscreen products able to protect the skin against damage from solar radiation requires safe and stable UV-absorbing molecules in formulas, which allow the uniform spreading of the UV-absorbing substances. A new generation of sunscreen products with high SPF values but low filter concentrations is the ultimate goal for researchers.
The aim of the present study was to determine the reason why a sunscreen spray, in which the concentration of UV-filter substances was twice as high as in the sunscreen cream, had the same SPF and also to demonstrate the role of fluorescent sunscreens as an innovative method to increase the efficacy of sun-protective products.

2 Materials and Methods

2.1 Volunteers

The investigations were carried out on 6 healthy volunteers aged between 25 and 45 years. Permission for the study had been obtained from the Ethics Committee of the Charité-Universitätsmedizin Berlin.

Pretreatment of the volunteers included washing of the skin with hand-warm water and drying with paper towels.

2.2 Sunscreen Samples

Samples of sunscreen cream and oil-free sunscreen spray were provided by Lancaster, Monaco. Both sunscreens had an SPF of 20 and contained the same mixture of UV-filter substances of Eusolex and Parsol. Only the concentration of UV-filter substances was different, with the spray having nearly double the amount (22% sun filter) compared to the cream (12% sun filter). The fluorescent dye fluorescein was added to part of both sunscreens at a concentration of 0.1% for the visualization of the homogeneity of the distribution of topically applied substances.

2.3 Application Protocol

The dye-containing cream and spray were applied onto an area of 4 cm × 4 cm on the forearm of 6 volunteers at a concentration of 2 mg/cm² in accordance with the COLIPA standard, respectively. The two sunscreens were applied using a syringe and were distributed homogeneously with a saturated glove finger as described previously.

2.4 In Vivo Determination of the Homogeneity of the Distribution of the Topically Applied Sunscreens

2.4.1 Laser scanning microscopy (LSM)

In a first step, the homogeneity of the distribution on the skin of both sunscreen cream and spray was measured in vivo with a confocal dermatological laser scanning microscope “Stratum” (OptiScan Ltd., Melbourne, Australia). The fluorescent dye in the sunscreen formulation was excited by an Argon laser radiation at 488 nm. The homogeneity of the dye distribution on the skin was analyzed on the forearm of the volunteers under in vivo conditions.

2.4.2 Tape stripping

In a second step, the homogeneity of the sunscreen distribution was analyzed ex vivo by tape stripping. The tape stripping procedure was performed as described previously. Following the application of the fluorescent dye containing sunscreens and the penetration time of 30 min, adhesive films (Tesafilm No. 5529, Beiersdorf, Germany) were pressed onto the skin with a roller and were subsequently removed. The tape strips were analyzed for the homogeneity of the distribution of the dye using fluorescent microscopy (BX60F3, Olympus, Deutschland GmbH, Hamburg, Germany).

2.5 Determination of the Spectroscopic Properties of the Sunscreens

Additionally, the sunscreen cream and spray (without fluorescein) were investigated for their spectroscopic properties. The absorption and fluorescence properties of the cream and the spray were analyzed in vitro using the UV/VIS-Spectrometer Lambda 40 and the Fluorescence Spectrometer, both from PerkinElmer, Ueberlingen, Germany. The fluorescence spectrometer was fiber-based, so the measurements were carried out in vitro in a cuvette and additionally in vivo on human skin after application of the sunscreens. In the latter case, a Wood light was used as an excitation source.

2.6 Investigation of the Radical Formation

The radical formation in the sunscreen spray and cream was analyzed after irradiation of the sunscreen samples with a sun simulator. For this purpose, an X-band electron spin resonance spectrometer (Galeniuss GmbH, Berlin, Germany) was used. The sunscreen samples were exposed to UV irradiation of 1 MED.

3 Results

3.1 Examination of the Homogeneity of Distribution of the Sunscreen on the Skin

The results regarding the homogeneity of the distribution of the topically applied sunscreens by laser scanning microscopy can be seen in Figs. 1(a) and 1(b). The distribution of the sunscreen spray and cream was observed to be nearly identical and had no important differences on living skin. The spray had a slightly better homogeneity of distribution, compared to the cream. Identical results were obtained for all 6 volunteers.

Utilizing the tape stripping technique revealed a slightly more homogeneous distribution of the fluorescent dye when applied as a spray in comparison to the cream, as shown in Figs. 2(a) and 2(b).

3.2 Investigation of the Energy Transfer Mechanisms of the Sunscreens on the Skin

The skin area treated with the spray showed a total absorption of the Wood light radiation (black area), whereas the area treated with the cream showed an intensive fluorescence (light area) (Fig. 3). The pink-colored fluorescent signal was analyzed using the fluorescence spectrometer.

Consequently, spectroscopic investigations regarding the fluorescence properties of 13 ingredients of the sunscreen cream were performed in order to identify the ingredient with the intensive fluorescent signal. Therefore, all compound ingredients of the cream, which were exclusively contained in the cream but not in the spray, were provided by Lancaster, Monaco, and were spectroscopically investigated for their fluorescence properties. It was found that only the compound ingredient Tetradodium EDTA had a fluorescent signal, identical to the spectral properties of the topically applied cream. This ingredient was not contained in the spray. Instead, the spray contained Disodium...
EDTA, which did not show any fluorescent signal. To substantiate that the compound ingredient Tetradodium EDTA was responsible for the fluorescent properties of the cream, a comparison between different combinations of the basic emulsion, the UV filters, and the ingredient Tetradodium EDTA was performed. The results are shown in Table 1.

3.3 Radical Formation
Using electron spin resonance spectrometric measurements, it was found that twice as many radicals had been formed in the sunscreen spray than in the sunscreen cream, whereas both spray and cream had been exposed to the same UV irradiation dose of 1 MED.

4 Discussion
New strategies for sunscreens that provide a wide coverage against sunlight are emerging. The present study was performed in order to clarify the reason why two sunscreens, a cream and a spray, with different concentrations of the same UV-filter substances, had the same SPF.

The protection efficiency of a sunscreen is determined by the absorption properties of the UV filter substances and by its homogeneity of the distribution on the skin. Consequently, in the first step the homogeneity of distribution of the cream and the spray was investigated by LSM and TS.

As both preparations provided the same SPF, a superior homogeneity was expected for the cream, as the cream contained only half of the UV filter concentration.
Contrary to expectations, the spray provided a slightly better homogeneity but with no significant difference from the cream.

These observations led to the conclusion that the spray should have a higher sun protection factor than the cream provided that the UV-filter absorption of the sunscreens is identical. However, as the SPF was identical, a further reason for the superior efficacy of the cream in comparison to the spray (same SPF with half of the filter concentration) has to be taken into consideration.

Therefore, the energy transfer mechanisms of both sunscreens were investigated using a Wood light. In this case, the spray showed a total absorption of the irradiation while the area treated with the cream showed an intensive fluorescence signal.

We hypothesized that the fluorescence properties of the sunscreen cream were caused by a single compound that was exclusively contained in the cream and provided an intensive fluorescent signal. Thereupon, a single compound only existing in the cream preparation could be identified providing an intensive fluorescent signal around 400 nm. As shown in Table 1, the emulsion alone and the UV filter substance plus the emulsion offered no fluorescent signal. The fluorescence intensity of the ingredient Tetradodium EDTA applied in the emulsion was 1 order of magnitude subordinate in comparison to the combination ingredient Tetradodium EDTA plus emulsion plus UV filter. Consequently, it can be assumed that an energy transfer from the UV filter to the ingredient Tetradodium EDTA takes place during UV irradiation. In the case of the spray, the UV-energy would be absorbed by the UV-filters and transferred into heat. It can be expected that during this process, free radicals are produced. Probably, these free radicals interact with the antioxidant protection system of our skin and stimulate the formation of an erythema, leading to a lower SPF. In the case of the sunscreen cream, it can be expected that the absorbed UV-energy is transferred into fluorescent light in the visible part of the spectrum, not harming the skin. Consequently, less UV-energy is transferred into heat and into radicals while the formation of an erythema is reduced, and a higher SPF is achieved.

Should this hypothesis be true, different amounts of radicals are produced during UV-radiation of the skin, in the case of the application of the sunscreen cream and sunscreen spray. Previously, it has also been demonstrated that some UV filter substances can stimulate the production of radicals when interacting with UV light.

Also in the present study, it could be demonstrated that—if the cream is exposed to UV irradiation—less free radicals are produced than in the case of the spray. This observation supports the hypothesis that the higher UV protection efficiency of the sunscreen cream is based on the nonthermal relaxation of energy and, consequently, reduced radical formation.

Taking into consideration the results of this study, it can be expected that the deactivation of the energy of the sunscreen after UV absorption of fluorescent light is a promising method to increase the efficiency of sunscreens.

In the case of the investigated sunscreen cream, the fluorescent light emitted after UV irradiation is not visible during daylight because it is emitted in the invisible part of the spectrum at a lower intensity than the solar radiation in this spectral range.

Additionally, it was demonstrated that optical methods could be efficiently used for the analysis of the homogeneity of the distribution of sunscreens on the skin and for the investigation of energy deactivation processes of sunscreens during irradiation.

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**References**


