Investigation of Model Sunscreen Formulations Comparing the Sun Protection Factor, the Universal Sun Protection Factor and the Radical Formation Ratio

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Key Words

Sunscreen efficacy · Chemical filter · Physical filter · Antioxidants · Human skin · Porcine ear skin · Electron paramagnetic resonance spectroscopy

Abstract

In view of globally rising skin cancer rates and harmful effects exerted by sunlight throughout the ultraviolet, visible and infrared ranges, an objective, safe and comprehensive method for determining sunscreen efficacy is required in order to warrant safe sun exposure. In this study, the influence of characteristic active ingredients (chemical filters, physical filters and antioxidants) on different sunscreen indicators, including the universal sun protection factor and the radical formation ratio, was determined and compared to their influence on sun protection factor values. Spectroscopic universal sun protection factor measurements were conducted ex vivo by analyzing tape strips taken from human skin, and radical formation ratio determination was performed via electron paramagnetic resonance spectroscopy using porcine ear skin ex vivo. The sun protection factor determination was conducted according to ISO standards (ISO 24444: 2010). It was shown that chemical filters provide a protective effect which was measurable by all methods examined

(spectroscopy, electron paramagnetic resonance spectroscopy and erythema formation). Physical filters, when used as single active ingredients, increased protective values in universal sun protection factor and sun protection factor measurements but exhibited no significant effect on universal sun protection factor measurements when used in combination with chemical filters or antioxidants. Antioxidants were shown to increase sun protection factor values. Radical formation ratio values were shown to be influenced merely by chemical filters, leading to the conclusion that the universal sun protection factor is the most suitable efficacy indicator for the ultraviolet range.

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Introduction

Changes in leisure activities and increasing skin cancer rates worldwide [1, 2] have led to a rising awareness regarding the importance of sun protection. While the acceptance of sunscreen use is surging, an objective, safe and comprehensive method for efficacy evaluation has become increasingly important.

Currently, the sun protection factor (SPF) is the accepted international standard for sunscreen efficacy eval-

uation [1–4]. Based on erythema formation, it is a measure of how long sun exposure can be prolonged before sunburn is induced. However, by employing erythema formation as a single efficacy indicator, one not only neglects the consequences of radiation in volunteer testing but also focuses on a single biological process, which is predominantly induced by ultraviolet B (UVB) radiation [1, 2, 5]. Damage known to be induced by ultraviolet A (UVA) and even infrared (IR) and visible (VIS) radiation [6–8] can only be insufficiently accounted for by applying the SPF. Hence, the development of new efficacy indicators is of utmost importance.

Based on different mechanisms, several authors have proposed new methods to determine sunscreen efficacy in a more comprehensive manner [9–11], inter alia, the universal SPF (USPF) [12] and the radical formation ratio (RF) [13], which is based on electron paramagnetic resonance (EPR) measurements.

The USPF permits the objective quantification of the protective efficacy of sunscreen products throughout the entire UV range. This method is based on the sum transmission spectrum that is determined spectroscopically by employing the noninvasive tape stripping procedure *in vivo*. The resulting sum transmission quantifies the reduction in the UV radiation intensity achieved by a product and forms the basis for the calculation of a new protection factor.

A different approach is offered by the RF determined via EPR spectroscopy. Based on the findings of Zastrow et al. [8] that free radicals are not only produced in the UV range but also up to 50% in the VIS and IR ranges, this method utilizes the underlying biological response of RF to determine the efficacy of sun protection, thus providing a possibility to determine the protective efficacy in the UV, VIS and IR ranges.

The aim of this study was to determine the influence of characteristic active ingredients on the USPF and RF and to compare them to the current sunscreen efficacy characterization standard, the SPF. While studies have shown reliable results for the EPR method in the IR and VIS ranges [14, 15], this study concentrated exclusively on the protection evaluation of these indicators in the UV range for comparative reasons.

For this purpose, commonly utilized active ingredients such as chemical filters (UVB and UVA filters), physical filters and antioxidant additives were added in defined amounts to different formulations and tested in a standardized setting.

The results provided an insight into the influence of different active ingredients on each efficacy indicator

tested, demonstrating the relation between them. Ultimately, the results helped to determine the most suitable efficacy indicator in the UV range as part of a safe, objective and comprehensive sunscreen efficacy evaluation.

Materials and Methods

Volunteers and Skin Samples

For the spectroscopic measurements, 30 healthy volunteers (22 female and 8 male) aged 21 through 36 (mean 25.5 years) with skin type II or III on the Fitzpatrick skin type scale were selected. Measurements were conducted on untanned skin. No skin diseases were reported and no scars or visible damage to the skin were observed.

The study was conducted in agreement with the Declaration of Helsinki. Consent was given by each volunteer investigated and permission of the Ethics Committee of the Charité – Universitätsmedizin Berlin was obtained. EPR measurements were conducted on 6 fresh porcine ears provided by a local butcher. Ethical approval was obtained from the Veterinary Board Dahme-Spree-wald, Germany.

Sunscreen Formulations

A total of 5 different formulations specifically prepared for the purpose of this study were used. For simplification purposes, the formulations were labeled with numbers 1, 2, 3, 4 and 5. The same base was used for each formulation (aqua, butylene glycol dicaprylate/dicaprate, glycerin, dioctylcyclohexane, polyglyceryl-2, dipolyhydroxystearate, glyceryl stearate, PEG-100 stearate, cetearyl alcohol, cetyl palmitate, magnesium aluminum silicate, xanthan gum, disodium ethylenediaminetetraacetic acid and preservative). The products only differed in their active ingredients: antioxidants (1% w/w; here bis-ethylhexyl hydroxydimethoxy benzylmalonate), chemical UV filters (12% w/w; a combination of butyl methoxydibenzoylmethane and octocrylene) and physical filters (2% w/w) consisting of titanium dioxide. The active ingredients of each cream are summarized in table 1.

The stability of the formulation was tested for 3 months at 40°C and with 10 cycles of freeze-thaw cycle, in which the formulation was frozen and thawed in a temperature interval of –5 to 40°C. Phenoxyethanol and ethylhexylglycerin were used as preservatives in the formulations.

Determination of the USPF

In line with ISO standards (ISO 24444:2010), 160 mg of sunscreen formulation was evenly distributed on a preferably hairless 8 × 10 cm skin area on the inner forearm of the subjects. After 60 min of penetration time, to obtain a stable and almost homogeneous distribution of sunscreen on the skin, a series of 10 tape strips (*tesa*® No. 5529; Beiersdorf, Hamburg, Germany) were taken from the same area of treated and untreated skin and immediately measured against an empty tape in the 240- to 500-nm range of a PerkinElmer Lambda 650 S UV/VIS spectrometer in front of the integrated sphere that was used in the transmittance mode. To obtain the absorbance capacity for each formulation, the influence of corneocytes on the spectra was corrected by subtracting the spectrum of the tape taken from untreated skin from the tape taken from treated skin using the UV WinLab program (UV WinLab

Table 1. Summary of the formulations and their active ingredients

Formulation	Active ingredients
Base	–
Cream 1	Antioxidants (1%; bis-ethylhexyl hydroxydimethoxy benzylmalonate)
Cream 2	Physical UV filters (2%; titanium dioxide)
Cream 3	Chemical UV filters (12%; butyl methoxydibenzoylmethane, UVA filter and octocrylene, UVB filter)
Cream 4	Chemical UV filters and antioxidants
Cream 5	Chemical UV filters, physical UV filters and antioxidants

version 6.0.3.0730; PerkinElmer GmbH, Rodgau, Germany) and the UV WinLab Data Processor and Viewer (PerkinElmer 2009 version 1.00.00.0010).

The resulting spectra were summated in an absorbance scale and later changed to a transmission scale to determine the sum transmission spectrum. The area under this spectrum quantifies the transmitted light in the complete UV range (280–400 nm) after the application of a formulation. The average UV sum transmission – related to 1 nm – was obtained by dividing the value of the area under the curve by 120, corresponding to the length of the complete UV range. This average sum transmission, which is given in percent, provided the basis for USPF calculations.

USPF = 100/average UV sum transmission in percent

The resulting USPF values indicated, similarly to the SPF, to what degree sun exposure may be extended before sun-induced injuries occur, with the distinction that USPF values account for radiation of both the UVB and UVA ranges. The applicability and the relevance of the USPF value were demonstrated by investigating a large number of commercial sunscreens from the lowest up to high SPF values [16].

Determination of the RF

Porcine skin samples were cleaned, shaved and dried using a paper towel before a punch biopsy (19 mm in diameter) was removed from an unscathed part of the ears. A filter disc was then placed on the biopsy and treated with 50 µl water:ethanol (1:1) 0.2% PCA solution (3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl; Sigma-Aldrich, Steinheim, Germany).

To avoid light-induced radical production, an occlusive and opaque covering was placed on the biopsy. The penetration time was set for 20 min. Subsequently, 5.7 mg (2 mg/cm²; ISO 24444:2010) of sunscreen formulation listed in table 1 was distributed homogenously on the sample. Following a 30-min induction period in an opaque chamber, the sample was placed in an L-band electron spin spectrometer (LBM MT 03; Magnettech, Berlin, Germany) and measured continuously for 16 min. Successively, the sample was exposed to UV/VIS radiation and measurements were recorded for another 16 min.

A solar simulator (LS0104; LOT, Darmstadt, Germany) containing a 150-watt Xenon arc lamp was utilized for this study. The corresponding settings have been described in detail by Meinke et al. [14]. The irradiation intensity was measured at 90 mW/cm². UVA was 8.55 mW/cm², UVB 1.75 mW/cm² and VIS 79.9 mW/

cm² (ILT 1400 Radiometer Photometer; Polytec, Waldbronn, Germany). After 16 min, the accumulated energy yielded 86.4 J/cm².

MPlot.exe was used for the EPR signal determination. Peak-to-peak measurements were conducted in the central line of the spectrum and measurements were normalized to the initial value to account for peak intensity variations between skin samples. The RF was then determined by establishing a ratio of mean signal intensity before and after UV/VIS irradiation.

RF = EPR signal before irradiation/EPR signal after irradiation

Determination of the SPF

The in vivo SPF was determined for 4 formulations (creams 2, 3, 4 and 5) by proDERM GmbH according to ISO standards (ISO 24444:2010). To determine the SPF values, each forearm of every volunteer was partly treated with the formulation and partly left untreated. After the penetration time, the volunteers underwent irradiation. Later on, both the treated and untreated areas were inspected for redness, and the minimal erythral dose (MED), which is the lowest UV dose that produces redness, was determined. The SPF was then calculated by dividing the MED of the protected skin by the MED of the unprotected skin, determining how long sun exposure can be extended before burning occurs.

Statistical Analysis

Statistical analysis of the results was performed using IBM SPSS Statistics version 20 and Microsoft Excel for Mac 2011. A p value of ≤0.05 was found to be statistically significant. A trend could be observed when p ≤ 0.1. The Kruskal-Wallis and Mann-Whitney tests were used to establish significant differences between the independent nonparametric mean values obtained.

Results and Discussion

By investigating the influence of the different models of creams on the selected efficacy indicator separately, the following results were obtained. Mean USPF values (fig. 1) were shown to be significantly increased by chemical filters present in the investigated formulations. The absorbance properties of the added filters led to a significant light attenuation. Furthermore, a small but statistically

Fig. 1. USPF value box plot for each tested formulation. AO = Antioxidants; PF = physical filter; c-UVF = chemical filter. * $p < 0.05$, significant difference between creams ($n = 6$ for each cream).

significant difference between cream 1, containing antioxidants, and cream 2, made with physical filters, was noted (1.05 and 1.24, respectively). On the one hand, this reflects the anticipated lack of effect of antioxidants as they did not influence the intensity of incident radiation, and on the other hand, it illustrates a small yet expected light attenuation due to the reflection and absorption properties of the physical filter. However, the observed protective efficacy of the physical filters could not be reproduced in measurements for creams also containing chemical filters. The lack of effect is attributable to the relatively small amount (2%) of physical filters added, resulting in a low extinction which does not remarkably influence the high values of the creams containing chemical filters. This can be explained by the much larger individual variation between USPF values for creams containing chemical filters, concealing smaller variations in light attenuation. Previous research has shown that increasing the doses of physical filters increases the protective efficacy of formulations [17], which is reflected in the USPF mean values. These results reinforce the use of the spectroscopic USPF as an objective measure of protective efficacy that is independent of biological processes and covers both the UVB and UVA ranges.

Figure 2 illustrates the RF after 16 min. EPR measurements show a significant difference between creams con-

Fig. 2. The RF after 16 min for each formulation. AO = Antioxidants; PF = physical filter; c-UVF = chemical filter. * $p < 0.05$, significant difference between creams ($n = 6$ for creams 1–4, $n = 7$ for cream 5).

taining no chemical filters (creams 1 and 2) and creams containing absorbing chemical filters (creams 3, 4 and 5). No significant differences between either creams 1 and 2 or creams 3, 4 and 5 were observed. This agrees with the small changes observed for the spectroscopic values of the corresponding creams. Contrary to expectations, antioxidants had no measurable statistically significant effect in the UV region. While previous research has shown the radical scavenging potential of topically applied antioxidants [18, 19] and has affirmed EPR spectroscopy as a suitable method for detection, this was not reproducible in this study. A possible explanation may be the relatively low amount of antioxidants added (1%) as well as the high ex vivo radiation intensity used in this study. In order to enhance the sensitivity of the measurements, a 20-fold MED was utilized in this setting, thereby possibly obliterating antioxidants early on.

Additionally, no effect of the physical filter was observed, which was not predicted. Previously, Meinke et al. [20] showed that not only antioxidants but also high scattering properties led to a significant reduction of radical formation in the near IR region. Physical filters primarily function by means of scattering and reflection, thereby preventing radiation from penetrating into deeper skin layers, and while free radicals may be produced on a smaller scale in the upper strata, excess free radical formation primarily

Fig. 3. SPF value box plot for each tested formulation. AO = Antioxidants; PF = physical filter; c-UVF = chemical filter. * $p < 0.05$, significant difference between creams ($n = 10$ for each cream).

produced in the deeper skin layers should be averted. Similarly to the small effects noted in USPF measurements, an explanation for the lack of effect may be provided by the low amount of physical filters present in the formulations (2%). Previous research employing a similar filter in a higher amount (5%) showed a substantial decrease of radical formation in the UV region, suggesting too little active ingredient for a measurable effect in this setting.

SPF testing was conducted by proDERM GmbH according to ISO standards (ISO 24444:2010). The results are summarized in figure 3. Chemical filters were again shown to be of clear protective value. Additionally, statistical testing disclosed a significant difference between cream 3, containing chemical filters as single active ingredients, and creams 4 and 5, which contained, in addition to the chemical filters, antioxidants (cream 4) as well as antioxidants and physical filters (cream 5). This had not been observed during USPF and RF testing, despite it being evidenced in previous research. It could be demonstrated that topically applied antioxidants exhibit protective effects during UV irradiation [21, 22], whereas previously, diets rich in antioxidants had been shown to reduce erythema formation [23]. As cream 1 was not tested, no conclusions regarding the protective effects of antioxidants independent of the presence of chemical filters could be drawn in this study. SPF testing for the physical

filter alone (cream 2) resulted in a mean protective value of 3.7. This is on par with our expectations as we estimated an increase of 2 SPF units for every percent of physical filter added.

However, previously described synergistic effects between physical filters and organic filters [24] could not be seen. The only cream containing both chemical and physical filters in addition to antioxidants did not provide a significant increase in the mean SPF value during testing when compared to cream 4, containing only chemical filters and antioxidants. No cream containing only physical and chemical filters was tested.

As Meinke et al. [14] pointed out, a possible explanation for this finding may lie within the antioxidant capacity of each cream. When the radical protection factor (which serves to express the antioxidative capacity in the creams) for these creams was determined, a much lower radical protection factor was seen for cream 5 $[(29 \pm 1) 10^{14} \text{ radicals/mg}]$ than for creams 1 $[(444 \pm 22) 10^{14} \text{ radicals/mg}]$ and 4 $[(459 \pm 28) 10^{14} \text{ radicals/mg}]$.

These results suggest that the antioxidant capacity may change when used in combination with physical filters. Hence, the SPF value seen in cream 5 is possibly a product of chemical and physical filters displaying the synergistic effects predicted rather than the antioxidants added in the cream. However, this hypothesis is contrary to the USPF values for creams 4 and 5, in which no synergistic effects of chemical and physical filters can be evidenced.

Lastly, efficacy indicators were analyzed for possible correlations. Although based on different mechanisms, due to the previously discussed lack of effect of antioxidants in the EPR measurements and little effect of physical filters in USPF mean values, the predominating protective effect was provided by chemical filters in both methods. From figures 1 and 2 it becomes obvious that high USPF values and low RF values were found for all creams containing chemical filters, suggesting a clear correlation between both efficacy indicators.

Conclusion

Chemical filters were shown to provide a protective effect in all three methods. Physical filters increased protection factors for USPF and SPF measurements as single active ingredients. However, they failed to show an effect when combined with chemical filters. Finally, antioxidants exhibited a protective effect during SPF testing.

In conclusion, the USPF is the most suitable efficacy indicator for both UV ranges (UVA and UVB). It is independent of biological damages, is determined in a noninvasive setting, objectively quantifies the protective efficacy of a sunscreen and is impartial to additives in formulations. Although the RF correlates significantly with the USPF in the current setting, it is less suited to evaluate sunscreen efficacy in the UV range. In this setting, high irradiation intensity had to be used to increase the sensitivity of the measurements, which therefore led to an obliteration of antioxidants early on, consequently preventing clear conclusions to be drawn regarding the protective efficacy of the antioxidants measured. Furthermore, the RF is based on a biological process susceptible to a variety of factors leading to changes in radical formation, such as the addition of supplemental anti-

inflammatory substances to a sunscreen, increased stress levels in the subject studied or differences in diet increasing or decreasing antioxidant capacity. Hence, it is less suited for an aspired objective evaluation in the UV spectrum.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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