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Commercial sunscreen formulations: UVB irradiation stability and effect on UVB irradiation-induced skin oxidative stress and inflammation

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ABSTRACT

Evidence shows that sunscreens undergo degradation processes induced by UV irradiation forming free radicals, which reduces skin protection. In this regard, the biological effects of three commercial sunscreen formulations upon UVB irradiation in the skin were investigated. The three formulations had in common the presence of benzophenone-3 added with octyl methoxycinnamate or octyl salycilate or both, which are regular UV filters in sunscreens. The results show that formulations *F*1 and F2 presented partial degradation upon UVB irradiation. Formulations *F*1 and F2 presented higher skin penetration profiles than F3. None of the formulations avoided UVB irradiation-induced GSH depletion, but inhibited reduction of SOD activity, suggesting the tested formulations did not present as a major mechanism inhibiting all UVB irradiation-triggered oxidative stress pathways. The formulations avoided the increase of myeloperoxidase activity and cytokine production (IL-1β and TNF-α), but with different levels of protection in relation to the IL-1β release. Concluding, UVB irradiation can reduce the stability of sunscreens, which in turn, present the undesirable properties of reaching viable skin. Additionally, the same SPF does not mean that different sunscreens will present the same biological effects as SPF is solely based on a skin erythema response. This found opens up perspectives to consider additional studies to reach highly safe sunscreens.

1. Introduction

Exposure to UV irradiation induces several skin responses such as inflammation, sunburn, pigmentation, vitamin D synthesis, immunosuppression, photocarcinogenesis, and photoaging [1]. UVB irradiation induces skin oxidative stress with the depletion of antioxidant defenses such as reduced gluthathione (GSH) and superoxide dismutase [2]. UVB irradiation also induces skin inflammation with the production of cytokines such as TNFα and IL-1β that recruit neutrophils [3,4]. There is a consistent interplay between cytokines and oxidative stress since cytokines induce the expression and activate enzymes such as NADPH oxidase, resulting in superoxide anion production. In turn, superoxide anion is also important for neutrophil recruitment and induces cytokine production [5–7]. Chronic UVB-induced skin inflammation and oxidative stress results in carcinogenesis [8,9].

To reduce skin photo-damage and the carcinogenic effects of solar irradiation, the use of sunscreen containing UV filters is recommended [10]. It has been shown that sunscreens can penetrate the skin and induce the production of reactive oxygen species (ROS) and that the use of sunscreen formulations may encourage prolonged sun exposure because they delay sunburn occurrence [11,12]. As erythema is the only *in vivo* response to UV irradiation considered for UV filters, it is possible that the lack of data regarding important biological responses triggered by UVB irradiation would provide an inadequate evaluation about UV filters protection against UVB-irradiation induced skin damages. Eventually, the limited parameters to allow a product to enter the market as a sunscreen is contributing to the use of products with limited efficacy over biological events crucial to skin diseases. In agreement with this possibility, the increased use of sunscreens has coincided with an increased incidence of skin cancer [13].

Several reports in the literature have shown that sunscreens undergo degradation processes induced by UV irradiation. This degradation of sunscreens leads to a reduction in the skin photoprotection capacity.

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Moreover, upon degradation there is formation of free radicals and other reactive/toxic intermediates that may directly or indirectly initiate damage in biologically relevant skin molecules [14,15].

Taking into account the all evidences mentioned above, the effect of three different SPF 15 commercial sunscreen formulations on skin under UV exposure was evaluated considering biological parameters. The three formulations had in common the presence of benzophenone-3 together with octyl methoxycinnamate or octyl salycilate or both, which are regular UV filters in sunscreens. Firstly, the present study assessed the photo-stability and the penetration of UV filters into the skin of hairless mice after the topical application of the three commercial sunscreen formulations. Finally, the activity of the SOD antioxidant enzyme, GSH levels, MPO activity, and IL-1β and TNF-α in the skin of animals treated with these commercial sunscreens and exposed to UVB irradiation were measured.

2. Methods

2.1. Sunscreen Formulations

Three commercial sunscreen formulations (*F*1, F2, and F3) currently available on the Brazilian market were investigated. The products were selected based on their equal SPF 15 value, but they each contained different photoactive substances, as indicated in their labels.

Formulation *F*1 contains tocopherol acetate, *Aloe barbadensis* and jojoba oil, in addition to the organic filters benzophenone-3 and octyl methoxycinnamate. Formulation F2 is composed of the organic UV filters benzophenone-3, butyl methoxydibenzoylmethane (avobenzone), octyl methoxycinnamate, octyl salicylate and homossalate. It also contains tocopherol acetate, licorice extract and beta-carotene. Finally, formulation F3 differs from *F*1 and F2 mainly due to the association of the organic filters benzophenone-3, bis-ethylhexyloxyphenol methoxyphenyl triazine, avobenzone, octyl salicylate, octyl triazone, and octocrylene with the physical UV filter, titanium dioxide. It also contains vitamin E.

2.2. Analytical Procedure

The amounts of benzophenone-3, octyl methoxycinnamate and octyl salycilate were determined by HPLC using a Shimadzu (Kyoto, Japan) liquid chromatography system equipped with an LC-10 AT VP solvent pump unit and an SPD-10 A VP UV–Visible detector operating at 305 nm. Separation was performed in a Supelcosil™ LC-18 HPLC column (25 cm × 4,6 mm, 5 μm) equipped with a precolumn C-18 filter (4 × 4 mm, 5 μm, Merck). This method was adapted based on methods already developed [16,17]. The mobile phase was methanol–water (84:16, *v*/v) containing 0.1% (*v*/v) acetic acid and was applied at a flow rate of 1 mL min^{-1}. The method was previously validated [18]. Benzophenone-3 (99.9%, Eusolex® 4360), octylmethoxycinnamate (99.9%, Eusolex® 2292), and octylsalicylate (99.8%, Eusolex® OS) standards were purchased from Merck (Darmstadt, Germany).

2.3. Photo-stability Studies: Absorption Spectra and Chromatographic Profiles

Samples of the *F*1, F2, and F3 formulations were spread onto a 20 cm^2 (approximately 2 mg/cm^2) area of a glass plate and left to dry for 30 min before exposure to UV irradiation (2.87 J/cm^2). The UV irradiation source was a Philips TL/12RS 40 W lamp (Holland-Medical). This source emits in the range of 270–400 nm with an output peak at 313 nm, resulting in an irradiation of 0.27 mW/cm^2 at a distance of 20 cm, as measured by an IL 1700 radiometer (Newburyport, MA, USA) equipped with UVB and UV detectors. The UVB output accounts for 78% of the total UVR. The same procedure was followed for the same length of time for the non-exposed formulations.

Subsequently, the exposed and the non-exposed formulations were diluted in ethanol to reach a final concentration of 0.2 mg/mL. To obtain the absorption spectra, the solutions were analyzed in a spectrophotometer in the wavelength range of 200–440 nm. The irradiation of formulations and the UV absorption measurements were performed in duplicate. The coefficients of variation of absorption measurements were <1.0% for all non-irradiated formulations, and <5.4, 3.5, and 9.8% for irradiated formulations *F*1, F2 and F3, respectively. Additionally, the filters benzophenone-3, octyl methoxycinnamate and octyl salycilate were quantified by HPLC analysis.

2.4. In vivo Skin Retention

In vivo experiments were performed on 3-month-old, sex-matched hairless mice. The animals, weighing 20–30 g, were housed in a temperature-controlled room, with access to water and food *ad libitum* until use. All experiments were conducted in accordance with National Institutes of Health guidelines for the welfare of experimental animals and with the approval of the Ethics Committee of the Faculty of Pharmaceutical Science of Ribeirao Preto, University of Sao Paulo (Process 10.1.811.53.1).

A total of 10 mg of the commercial sunscreen formulation (*F*1, F2 or F3) was applied on the backs of the animals in a delimited area of 1.77 cm^2. One hour after the application of the formulations, the animals were sacrificed by inhalation of carbon dioxide, and the treated skin area was removed and subjected to the process of tape stripping. Tape stripping allowed determining UV filters only in viable skin.

The retained UV filters in the viable epidermis plus the dermis were extracted with methanol, and the resulting solution was then filtered using a 0.45 μm membrane. BP-3, OMC and OS were then assayed by HPLC as described previously in the analytical procedure item 2.2 [15].

2.5. UV Irradiation

Mice were divided into five groups with five animals per group: Group IC = irradiated control, Group NIC = non-irradiated control, Group *F*1 = treated with *F*1 and irradiated, Group F2 = treated with F2 and irradiated, and Group F3 = treated with F3 and irradiated.

The treatment protocol consisted of topically applying 30 mg of the formulations on the back of the animals 1 h before the irradiation. The groups exposed to UV irradiation were placed in a wooden enclosure containing the same lamp used in the photo-stability studies and irradiated for 3 h, which corresponds to a dose of 2.87 J/cm^2. Mice were euthanized with an overdose of carbon dioxide 3 h (for MPO, TNF-α, and IL-1β evaluation) or 6 h (for GSH and SOD determination) after the end of the UV exposure, and full dorsal skins were removed and stored at −80 °C until analysis [15,19,20].

2.6. In vivo Photo-protection of Commercial Formulations

2.6.1. GSH Levels

GSH levels were determined using a fluorescence assay [21]. The collected skin (1:3, *w*/w dilution) was homogenized using Ultra-Turrax (IKA®, Germany) in 100 mM NaH_2PO_4 (pH 8.0) containing 5 mM EGTA. The homogenates were treated with 30% trichloroacetic acid and then centrifuged at 1900*g* for 6 min. After that, 100 μL of the supernatant was mixed with 1 mL of 100 mM NaH_2PO_4 (pH 8.0) containing 5 mM EGTA and 100 μL of o-ftalaldeide (OPT) (1 mg/mL in methanol). The fluorescence was determined after 15 min (k exc = 350 nm; k em = 420 nm) and the values were obtained by comparing to a standard curve of GSH. Results are presented as μmol of GSH per mg of skin [3].

2.6.2. SOD Activity

SOD activity was determined in epidermal extracts according to the reduction of ferrycytochrome *c* method [22]. The total skin (1:2, *w*/w