A NOVEL APPLICATION OF THE EPISKIN PHOTOTOXICITY ASSAY (EPA)

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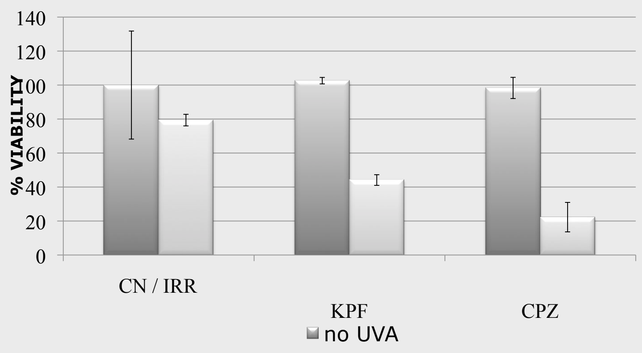
BACKGROUND AND AIM

Phototoxicity is an inflammatory, non immunologically mediated, light induced skin reaction after contact with a phototoxic compound. The substance may reach the skin following topical application or after systemic administration via the blood flow. It is known that the validated Balb 3T3 NRU method (OECD 432) may provide an over- prediction of photo-toxicity of chemicals due to the lack of penetration barrier. The Episkin Phototoxicity Assay, EPA has defined the relevance of the Episkin model to discriminate between Phototoxic and non phototoxic ingredients compared to the 3T3 NRU and moreover the possibility to assess the systemic photo-toxicity has been successfully evaluated on reference molecules. Within pharmaceutical and dermo-pharmaceutical industries there is a need for testing the efficacy of products formulated to protect the skin of patients users of photo-allergens or phototoxic substances (Chlorpromazine-CPZ, antibiotics, FANS as Ketoprofen-KPF) during their occasional exposure to the sun light. These products are currently called photo-protectors and they may or may not contain UV filters and be based on different mechanism of photo-protective action.

Aim of this work has been to assess the relevance of the EPA assay as a **photo-toxicity model** induced by the systemic exposure to reference phototoxic molecules (KPF and CPZ), in order to discriminate the protection offered by topically applied products that may contain antioxidants, mineral pigments and UV filters.

RESULTS

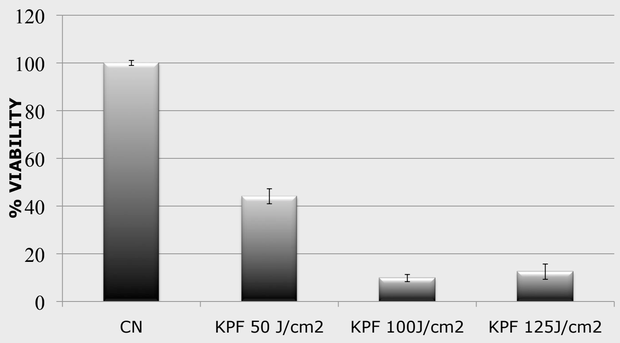
PROCEDURE VALIDATION ASSAY



The systemic induced photo-irritation has been assessed by using two reference molecules, CPZ and KPF added in the medium underneath the Episkin model at previously defined dose.

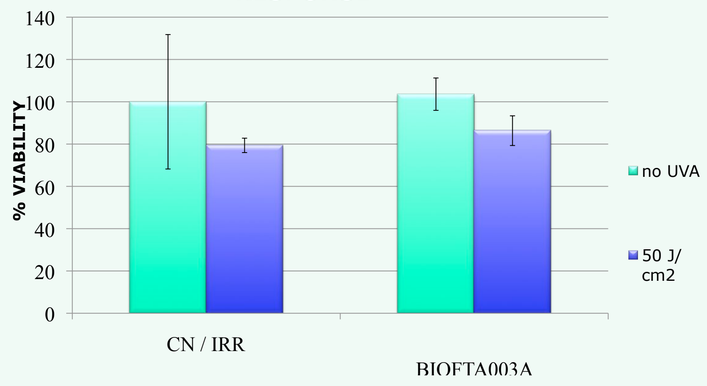
The irradiation dose was 50 J/cm2. After treatment with both KPF 0,15 mM and CPZ 0,2 mM the observed decrease in cell viability was >25%. In parallel, MTT test was performed without UVA to test the cytotoxicity of the chemicals.

SYSTEMIC KPF : UVA DOSE EFFECT



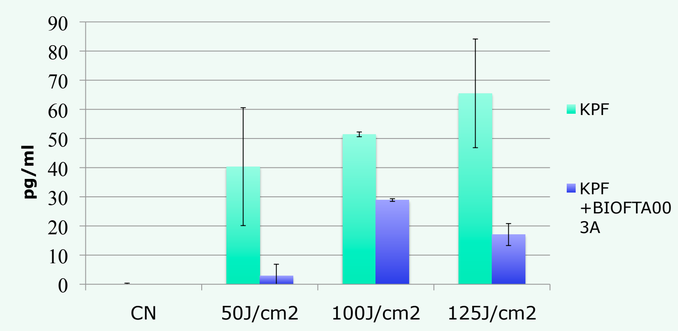
SYSTEMIC KPF : UVA DOSE EFFECT RESPONSE
Episkin model has been exposed up to 100 and 125 J/cm2 UVA doses with no cytotoxic effects. After treatment with KPF the observed decrease in cell viability was >25%.

ASSESSING BASELINE PHOTO-TOXIC RESPONSE



The skin irritation potential of the product BIOFTA003A has been assessed by the MTT viability test after 50 J/ cm ^{2} and without UVA in the same exposure adopted in the EPA assay: any intrinsic photo-toxic potential has been quantified.

IL-1 ALPHA RELEASE



KPF treatment, as expected, induced a release of IL-1α that was UVA dose dependent and constantly over 40 pg/mL.

At 50, 100 and 125 J/cm^2 the release of IL-1α after treatment with product BIOFTA003A was increased but below the cut off of the EPA prediction model: any inflammatory reaction could be attributed to the product

EPA-KPF MODEL : PHOTO-PROTECTIVE EFFICACY OF BIOFTA003A

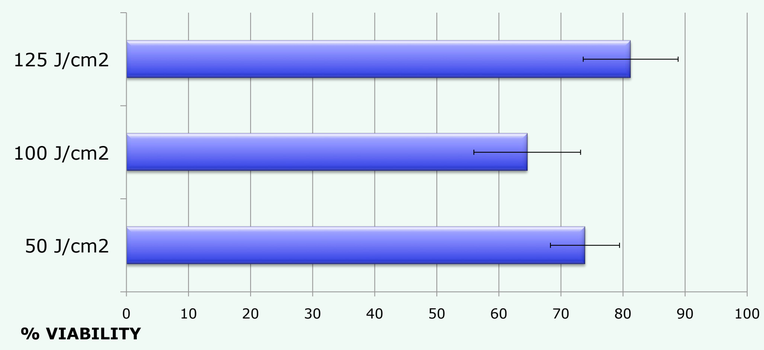
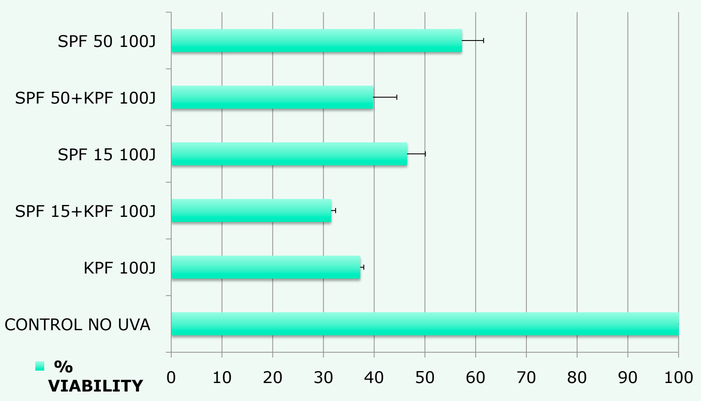


PHOTO PROTECTIVE EFFICACY AGAINST KPF-INDUCED PHOTOTOXICITY

The product BIOFTA003A topically applied was efficient in counteracting the phototoxic reaction induced by the KPF: the residual viability after 50J - 100J - 125 J/ cm^2 was > 60% .
The protection offered by the sun products containing UVA and UVB filters has been quantified as similar (SPF 50) and lower (SPF 15).

EPA-KPF MODEL: PHOTO-PROTECTIVE EFFICACY OF SUN PRODUCTS



IRRADIATION SYSTEM

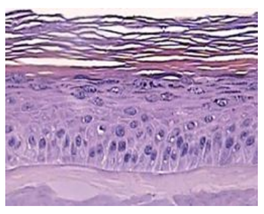
A 1 kW Oriel solar simulator equipped with Spectra-Physics Lamp (Xenon 1000W), 3 Schott WG335 / 1 mm filter emitting UVA and visible light but no UVB radiation was used for all irradiation experiments.

IL-1α QUANTIFICATION

IL-1α release in the culture medium was determined by quantitative sandwich enzyme immunoassay technique (Quantikine, R&D Systems, UK).

IN VITRO TISSUE CULTURE : EPISKIN MODEL

The EpiSkin model (EPISKIN S.N.C., Lyon, France) is a reconstructed organotypic culture of human adult keratinocytes reproducing a multilayered and differentiated human epidermis SkinEthic Laboratories are gratefully aknowledged for the free Episkin models provided for this research study.



EXPERIMENTAL DESIGN

EPA prediction model

Phototoxic classification of test-chemicals was evaluated by using a decrease of cytotoxicity cut-off >25% and an IL-1-α release cut-off of 40pg/mL.
UVA-absorbing chemicals are classified as phototoxic after topical or systemic-like treatment if they are significantly above these limits.

In vitro EPA (EpiSkin Phototoxicity Assay)

Following overnight incubation at 37°C upon reception, epidermis were transferred to 12-well plates containing 1.5mL of PBS (Ca2+& Mg2+). 100µL of KPF or CPZ were added to the underlying culture medium (systemic-like administration) and placed at 37°C for 2 hours. After 2 hours of incubation, 100µL of formulations (BIOFTA003A cream without UVB filters and SPF Value but containing only UVA filters, SPF 15 +UVA lotion, and SPF 50+UVA cream) has been topically applied on Episkin and then exposed to 50 J/ cm^2 up to 125 J/cm^2 dose. In parallel, the non-irradiated control tissues were placed in the dark under the same conditions. After a 2 hours post-irradiation period, tissues were incubated overnight at 37°C, 5% CO_2 and 95% humidity in fresh maintenance medium. Cytotoxicity by MTT test and IL-1α determination on culture media of Episkin models were quantified.

CONCLUSION

The results have confirmed the phototoxic potential of Ketoprofene 0,15 mM and CPZ 0,2 mM after *systemic like* application and the protection offered by topical formulation containing UVA Filters, antioxydants and cytoprotective agents. The EPA assay was useful to assess the efficacy of the product BIOFTA003A and sun products with low and high Sun Protection Factors (15 and 50 respectively) against photo-toxic KPF induced skin reactions with different doses of UVA. Compared to the sun products the product BIOFTA003A was more efficient than an SPF 15 and gave similar results to SPF 50 in counteracting the photo-toxic reaction induced by systemic KPF.

REFERENCE
Lelievre D et al. The Episkin Phototoxicity assay (EPA): development of an in vitro tiered strategy using 17 reference chemicals to predict phototoxicpotency. Toxicology in Vitro 21 (2007) 977-995.

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IVTIP Meeting with ESTIV and CAAT April 26-27, 2011, Monaco

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