Photoprotective complementary effects of sun filters and a combination of active molecules on UV-exposed human volunteers

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Introduction

Chronic exposure to ultraviolet (UV) irradiation causes immunosuppression, photoaging, and carcinogenesis [1] by induction of a cascade of skin damage. Although sunscreens with a very high sun protection factor (SPF) absorb most of the sun's UVB rays, no sunscreen is effective in reducing total UV effects, particularly those induced by UVA. In the **context of an ecobiological approach** where skin natural resources and mechanisms must be preserved, and thus **to increase UVA protection, UV filters and antioxidants have been combined** to enhance their photoprotective effect, but studies in humans are lacking. The present study aimed to characterize the photoprotection properties of an **association of ectoine and mannitol** and **to investigate its additional photoprotection effect** in humans in combination with UV filters based on the assessment of a **UV-induced biomarker, the oxidized squalene**.

Materials & Methods

➢ *In vitro protocol*

Cell culture NHEK were pre-incubated for 24 hours with the active association (0.01% ectoine and 0.01% mannitol). After incubation with the fluorescent probe 2,7-DCDHF-DA, the culture plates where the SPF30 filters were applied (1 mg/cm2) were left as is or covered with a quartz plate.

Irradiation The cells were left untreated or irradiated with UVB at 100 mJ/cm² and UVA at 0.7 J/cm² using a SOL500 Sun Simulator equipped with an H2 filter.

Quantification of oxidative stress After irradiation, the cells were incubated for 30 minutes in PBS with and without the active association (0.01% ectoine and 0.01% mannitol) before quantification of the production of ROS by measurement of the fluorescence emitted by the 2,7-DCDHF-DA probe (λex = 485 nm, λem = 538 nm) using a microplate reader (Fig. 1).

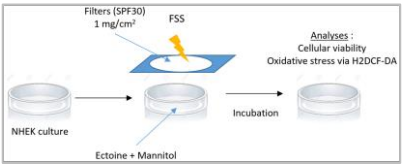

Figure 1 : Scheme of the *in vitro* H_2DCFDA protocol

➢ *In vivo protocol*

Volunteers This study was conducted on 10 male volunteers aged from 20 to 44 years, with oily skin and a cutaneous sebum rate ≥30 µg/cm² (quantified using Sebumeter®). The samplings were performed on the back of volunteers by swabbing, a non-invasive technique.

Protocol timeline At D0, a skin surface sampling was performed to determine the basal value of squalene for each subject, and their own MED was determined. **From D0 to D2 included (3 days), an application of the studied products** was performed at the clinical centre by the technician (**2mg/cm², twice daily**), according to the 4 studied zones defined on the back of volunteers: placebo; sun filters SPF 30; the active complex containing 0.1% ectoine and 0.1% mannitol; sun filters SPF 30 in association with the active complex. At D3 morning, after a standardized cleansing and a last products application (30 minutes before irradiation), **a 2 MED UV exposition** (UVA and UVB) was then performed on the 4 selected UV sub-zones for all conditions with a xenon lamp Monoport - 300W (Solar Light). **At D4, skin surface samplings** were done on the 8 sub-zones of each volunteer (4 irradiated and 4 non-irradiated) and stored at -20°C.

Biochemical analyses Squalene oxidation (quantity of oxidized squalene and non-oxidized squalene) was measured by HPLC system Ultimate 3000 coupled with a single quadrupole mass spectrometer detector ISQ (Thermo Scientific).

Statistical analysis For each parameter, normality was first checked to determine the right statistical test to applicate. If hypothesis of normality was approved, a Student's t-test was used, and if not, a Wilcoxon test. If the p-value was less than 0.05, the difference was significant.

Results & Discussion

➢ *In vitro efficacy of the active association with and without UV filters on UV-induced oxidative stress*

The intracellular oxidative stress was induced by UVs by 2.5-fold change (Fig. 2). This stress was significantly reduced by the sun filters alone by 51%.

The active complex (0.01% ectoine and 0.01% mannitol) increased the antioxidant protection provided by filters by 35%. The **combination of sun filters with this active complex** attributes to the finished product up to **86% antioxidant protection**.

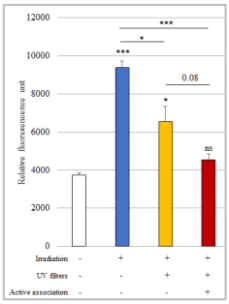

Figure 2 : *In vitro* assessment of intracellular ROS production quantified by H_2DCFDA fluorescence in NHEK. *p<0.05, ***p<0.001; ns=not significant.

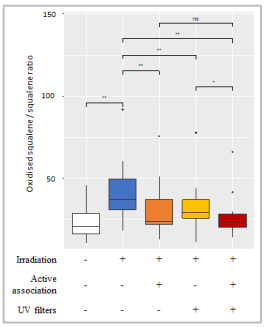

Figure 3: *In vivo* quantification of the oxidized squalene/squalene ratio. The results are presented as box plots, with whiskers representing the maximum values or 1.5 times the interquartile range of the data, whichever was smaller. Wilcoxon's signed-rank test for squalene. *p<0.05, **p<0.01, and ***p < 0.001; ns=not significant.

➢ *In vivo efficacy of the active association with the UV filters after UV-irradiation on oxidative stress*

UV irradiation Compared to non-irradiated vehicle-treated areas, UV irradiation induced a significant 2-fold (p<0.01) increase in the oxidized squalene/non-oxidized squalene ratio (Fig. 3).

Ingredients alone Compared to the irradiated vehicle-treated areas, the active association and UV filters alone protected squalene oxidation by 58.4% (p<0.01) and 50.6% (p<0.01), respectively.

Combination of the active association with UV filters provided the best level of protection in terms of squalene oxidation (**76.8%;** p<0.01) compared to the irradiated vehicle-treated areas. Compared to UV filters alone, a **significant additional protection of squalene by 26%** (p<0.05) was observed.

Following the **principle of ecobiology**, which is an original approach considering the skin as an ever-evolving ecosystem whose natural resources and mechanisms must be preserved [2, 3], **the association of ectoine and mannitol combined with the UV filters protects skin components and preserves the skin antioxidant system**.

The main limitation of this work is the small sample size of subjects in the *in vivo* study. A study including more subjects and investigating other sun protection biomarkers could be interesting to provide further evidence of efficacy and thus to understand more globally the photo-protective mechanisms involved.

Conclusions

In conclusion, our study performed ***in vitro*** **as well as** ***in vivo*** with **non-invasive analysis of biomarker** showed that this **active association provides significant additional skin photoprotection to UV filters in terms of reduction of oxidative stress induced by UV rays**. Sunscreens containing active ingredients, appear to provide better photoprotection at a cellular level than UV filters alone, and this ecobiological approach should be taken into consideration by dermatologists and users of such products.

Acknowledgements

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References

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