COMPLEMENTARY PHOTOPROTECTIVE ANTIOXIDANT EFFECT OF SUN FILTERS AND A COMBINATION OF ACTIVE MOLECULES IN A MODEL OF IRRADIATED HUMAN KERATINOCYTES

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BACKGROUND

Skin photoprotection has become a real public health issue in view of consequences of the sun on unprotected skin such as erythema, immunosuppression, skin cancer. The major role of UVA rays has long been neglected, even though their harmful effect over the long term, by notably generating oxidative stress. To prevent it, it has become important to provide biological protection in addition to sun filters.

The aim of this study was to demonstrate the *in vitro* complementary efficacy of an active complex (ectoine and mannitol) with sun filters on intracellular oxidative stress in normal human epidermal keratinocytes (NHEK) irradiated with the full solar spectrum (FSS).

MATERIAL & METHODS

- Cell culture NHEK were pre-incubated for 24 hours with the combination of ectoine and mannitol at 2 doses (n=3). Then, the formula containing sun filters (SPF30) was applied (1 mg/cm²) on a quartz plate placed above the culture plates.
- Irradiation Cells were then irradiated with the FSS (calibrated on UVB 100mJ/cm2 + UVA 0,7 J/cm²) with a SOL 500 Sun Simulator equipped with H2 filter.
- Quantification of oxidative stress The intracellular reactive oxygen species (ROS) production was then evaluated 30 minutes post-irradiation with the fluorescent probe H_2DCF-DA.

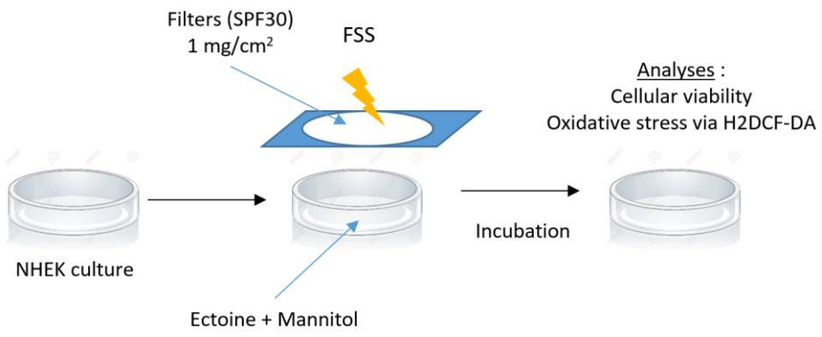

Figure 1: Scheme of experimental protocol

Control conditions without cream were performed in parallel. Fluorescence levels quantified with a spectrophotometer (λex= 485 nm, λem= 538 nm) are proportional to the quantity of intracellular ROS. A control of cell viability was performed on cell layers using a MTT reduction protocol.

- Statistical analyses The data were collected from one experiment carried out in triplicates. Quantitative analyses are expressed as mean ± standard deviation. Statistical significance is determined by a Student's test. Differences are considered statistically significant as from p<0.05 (NS = p>0.05; * = p<0.05; ** = p<0.01; *** = p<0.001).

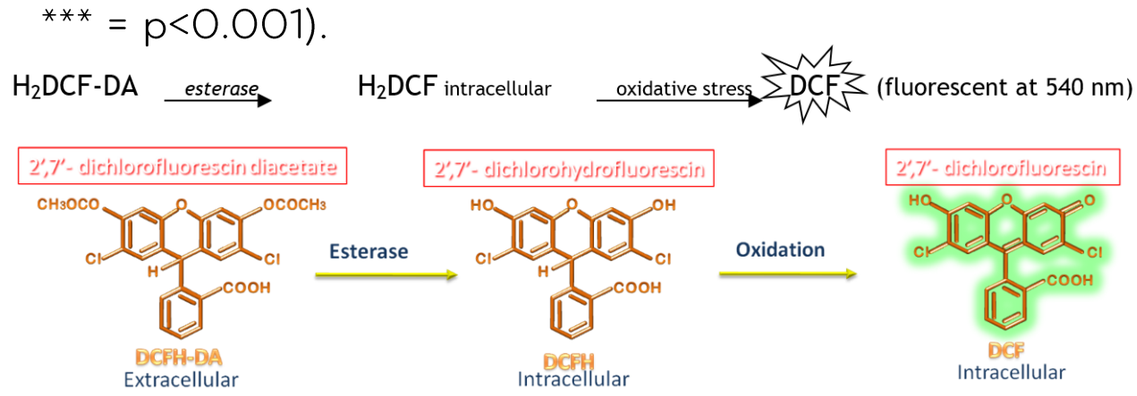

Figure 2: Principle of H_2DCF-DA assay

RESULTS

- UV irradiation The intracellular oxidative stress was induced by FSS by 2.5-fold change.
- Sun filters This stress was significantly reduced by the sun filters alone by 51%.
- Sun filters with ectoine and mannitol The active complex increased the antioxidant protection provided by filters, by 31% for the 0.003% ectoine and 0.003% mannitol condition and by 35% for the 0.01% ectoine and 0.01% mannitol condition. The combination of sun filters with this active complex attributes to the finished product until 86% antioxidant protection.

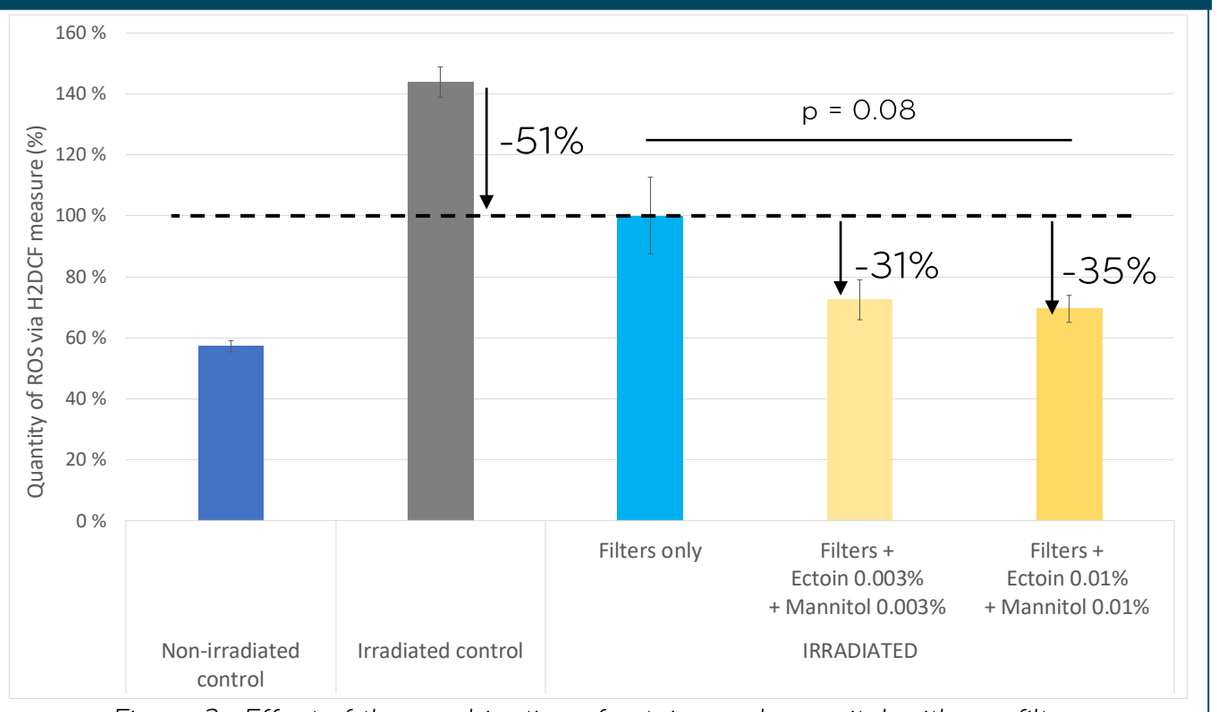

Figure 3 : Effect of the combination of ectoine and mannitol with sun filters on intracellular oxidative stress after 30 minutes post-irradiation

CONCLUSION

This study demonstrates the interest of combining UV filters with biological photoprotection to prevent oxidative stress generated by UVA damage after sun exposure.

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