Photoprotective effect of ectoine and mannitol to preserve the skin according to ecobiology approach.

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Sylvie Callejon[1], Baba Teme[1], Sophie Weber[1], Benoît Cadars[2], Marlène Chavagnac[2], Fabienne Alfonsi[2], Sandra Trompezinski[1], Aurélie Guyoux[1]

[1] NAOS, Aix-en-Provence, France
[3] NAOS / Bioderma, Lyon, France

BACKGROUND

Exposure of skin to UV irradiation causes oxidative stress, DNA damage and immune suppression associated with increased incidence of skin cancer. Therefore, protection against UV-induced damages may have a positive skin impact.

First, intracellular reduced glutathione (GSH) is an intracellular tripeptide that neutralises reactive oxygen species. GSH is depleted with UVA irradiation[1].

Then, UVA-induced DNA double-strand breaks results from the repair of clustered oxidative DNA damages[2]. The phosphorylation of H2Ax is a marker of DNA double-strand breaks.

Finally, the immunosuppressor effect of UVA was revealed through a reduction of the Langerhans cells (LC) ability to present antigen followed by a reduction of the T lymphocytes (TL) proliferation.

To assess the effects of active compounds, three in vitro tests were performed on protection of GSH level, H2Ax phosphorylation and Langerhans cells (LC) ability to present antigen.

MATERIAL & METHODS

Quantification of intracellular reduced glutathione (GSH) :

HaCaT keratinocytes were pre-treated for 24 hours with DMEM containing ectoine (Merck) and mannitol (IMCD). Then, cells were irradiated with full solar spectrum (200J/cm², 750W/m²) in HBSS in the absence of active ingredients. After irradiation, the cells were incubated for 24 hours in culture medium before measuring the GSH by ELISA (V6911, Promega).

DNA damage scoring :

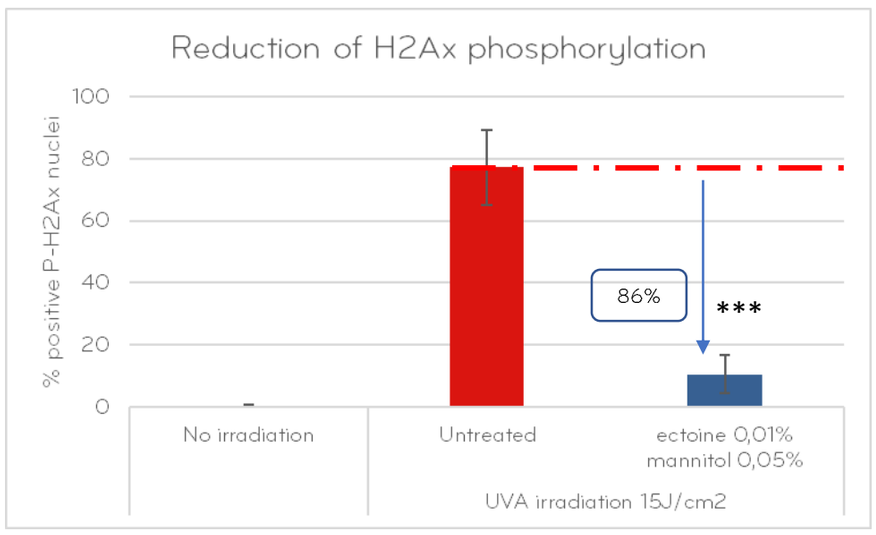
Normal Human Epidermal Keratinocytes were grown in supplemented keratinocytes growth medium 2 (Promocell) at 37°C and 5% CO2. 1 day after plating, cells were treated with 0.01% ectoine and 0.05% mannitol. Next day cells were irradiated with 15 J/cm² UVA (Waldmann tubes PL 36W/09 PUVA) for 1 hour and fixed for further treatment.

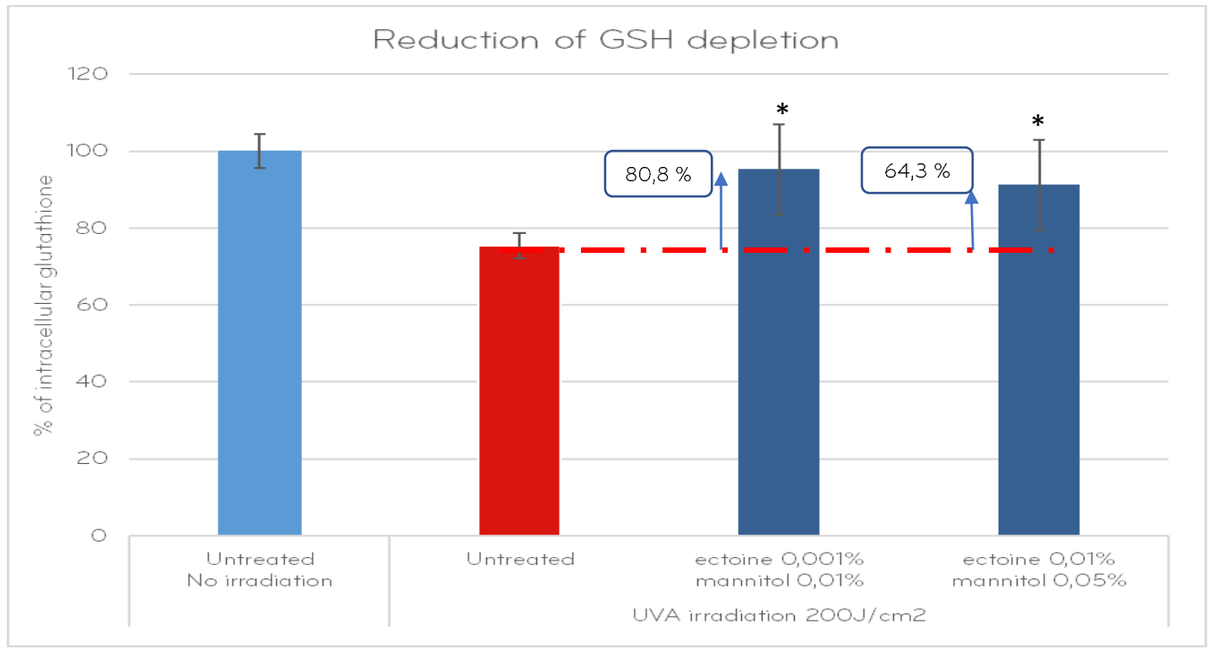
DNA lesions were scored using an immunolabelling of histon 2 phosphorylation on S139 and fluorescence imaging on an Olympus BX-60 microscope. Nuclei positives for H2Ax was compared to total population of nuclei (stained using DAPI).

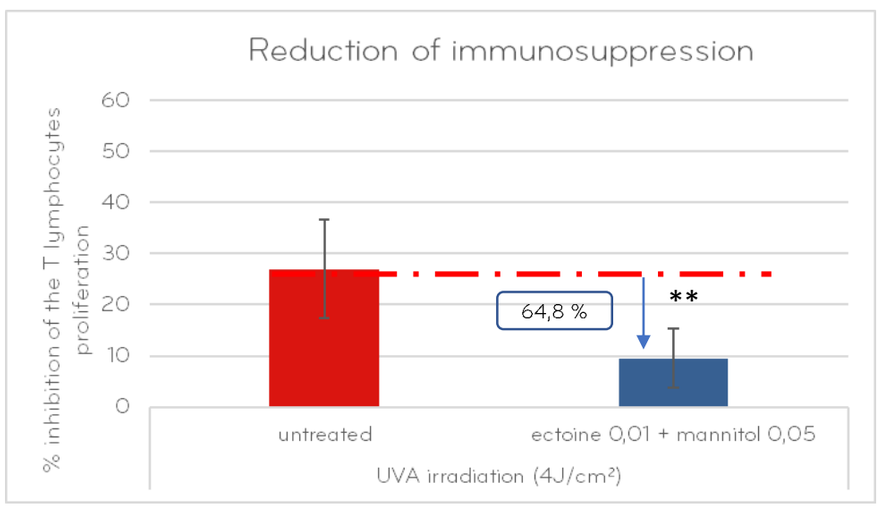
Evaluation of the Langerhans cells (LC) functionality :

After purification from human skin biopsy, LC were incubated for 18 hours in RPMI-1640 supplemented with 10% FBS, GM-CSF (200ng/ml) and ectoine and mannitol. Then, cells were irradiated with 4J/cm² UVA 365 nm. After irradiation, LC were cocultured with TL in RPMI-1640 with 10% human serum AB. TL were isolated from human blood. TL proliferation was measured after 5 days with tritiated thymidine. Radioactivity was measured with β counter.

RESULTS







Ectoine and mannitol complex :

- restricted GSH UV-induced depletion by 80.8 % ($p<0.05$)
- decreased the H2Ax phosphorylation by 86 % ($p<0.001$).
- increased T lymphocytes proliferation by 64.8 % ($p<0.01$) indicating the partial maintain of antigen presentation capacity of LC.

Statistical analysis :

A statistical analysis by a Student test was performed. All conditions were carried out in triplicates. Differences are considered statistically significant from $p<0.05$ (NS : $p>0.05$; * : $p\leq0.05$; ** : $p\leq0.01$; *** : $p\leq0.001$).

CONCLUSION

Our results demonstrate that ectoine and mannitol help the skin preserve the intracellular machinery offsetting the deleterious effects of sun irradiation by maintaining GSH levels as well as by reducing H2Ax phosphorylation and immunosuppression. The mode of action of this complex follows the principles of ecobiology, a novel scientific approach founded on the premise that the skin is a constantly changing ecosystem interfaced with its environment and this interaction is based on a host of natural resources and mechanisms which must be preserved.

1. Cadet J, *et al.*, Sensitized formation of oxidatively generated damage to cellular DNA by UVA radiation. Photochem Photobiol Sci. 2009 Jul;8(7):903-11.
2. Greinert R, *et al,* UVA-induced DNA double-strand breaks result from the repair of clustered oxidative DNA damages. Nucleic Acids Res. 2012 Nov 1;40(20):10263-73.
3. Halliday G M *et al,* UVA-induced immunosuppression, Mutat Res, 1998; 422: 139-145

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