Conclusions

- The results of this study demonstrate that a single application of the photostable UVA/UVB spectral absorbent sunscreen protects skin against a harsh UV insult (30 MED).
- Protection was provided for multiple markers and the changes induced by the sunscreen treated 30 MED-insult were not significantly different than by the low level exposure (1 MED) necessary to cause the minimum erythema.
- Strong *in vivo* photostability of the test sunscreen is indicated by its protective capacity following the intense 30 MED exposure.

References

1. Walker, S. L., Hawk, J. L. M., Young, A. R. *Acute and Chronic Effects of Ultraviolet Radiation on the Skin.* In: Freedberg, I. M., Eisen, A. Z., Wolff, K., Austen, K. F., Goldsmith, L. A., Katz, S. I., editor. Fitzpatrick's Dermatology in General Medicine. 6th ed., New York: McGraw-Hill Inc., 2003
2. Kullavanijaya, P., and Lim, H. W. Photoprotection, *J. Am. Acad. Derm.,* **52(6)**:937-958, 2005
3. Cole, C., Chen, T. and Appa, Y. *Comparison of Photoprotection Efficacy and Photostability of Broad Spectrum Sunscreens.* Poster Presentation at the 64th Annual Meeting of the American Academy of Dermatology in San Francisco, March 2006.

Evaluation of Protective Effect of A Sunscreen Against UV Induced Cellular Damage

TECHNICAL BULLETIN

Neutrogena Dermatologics

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Introduction

There is a widespread acceptance that unprotected ultraviolet radiation exposure causes prematurely aged skin and can cause skin cancer.[1] There is, however, less public awareness regarding the epidermal and dermal damage of UV at the cellular and molecular level. The immunosuppressive effects of UVB have been documented and recent work has indicated that UVA radiation may be even more immunosuppressive.[2] Thus, daily protection against UV exposure should prevent not only photoaging, but protect against changes leading to photoimmunosupression and potential skin cancers.

A patented sunscreen system combining avobenzone and oxybenzone and the photostabilizer diethylhexyl 2,6-naphthalate has been developed to provide exceptional absorbance throughout both the UVA and UVB spectrum with maximum photostability.[3]

Objective

The objective of this study was to assess the capability of this technology to inhibit the formation of UV induced immunological and cellular markers representing multiple pathways of skin damage.

Materials and Methods

- In this study, 11 healthy participants (2 male, 9 female), ranging in age from 20 to 48 years (mean 34.6), completed participation to evaluate the effectiveness of a full-spectrum SPF sunscreen containing actives avobenzone, homosalate, octisalate, octocrylene and the stabilizing agent diethylhexyl 2,6-naphthalate
- Following determination of each subject's minimal erythema dose (MED), four test sites on the lower back were treated as follows:
  - **Treated site** (2 mg/cm^2): Irradiated with 30 MED
  - **Untreated site** (positive control): Irradiated with 1 MED
  - **Untreated site** (negative control): Not irradiated
- Twenty-four hours after exposure, using standard procedures, skin biopsies were taken, stained and assessed for histological changes by a board certified pathologist.
- Histologic parameters
  - **MMP1 and MMP9** (MMP immunohistochemical stain) – evaluated on a 0 – 4 scale (0 = no or minimal expression within keratinocytes and 4 = marked diffuse expression within all keratinocytes)
  - **Mast cells** (tryptase immunohistochemical stain) – cells exhibiting strong cytoplasmic staining per square mm of epidermis
  - **Sunburn cells** (H&E stain) – number of keratinocytes with dense hypereosinophilic cytoplasm and dark small picnotic nuclei per mm of epidermis.
  - **Thymine dimers** (thymine dimer immunohistochemical stain) – evaluated on a 0 – 4 scale (0 = 0% of keratinocytes with nuclear staining and 4 = 76%-100% with nuclear staining)
  - **Microvessels** (CD31) – positive staining microvessels with visible lumen per square mm of epidermis.
  - **Microvessels Dilatation** – evaluated using a 0 – 4 scale (0 = no dilatation/all vessel lumens ≤ 14 microns and 4 = severe/≥10% of vessel lumens 55 microns)
  - **Mast cells** (tryptase immunohistochemical stain) – cells exhibiting strong cytoplasmic staining per square mm of epidermis
  - **Langerhans cells** (CD1a) – cells within the epidermis exhibiting moderate to strong positive staining cytoplasm and a visible nucleus per mm of epidermis.

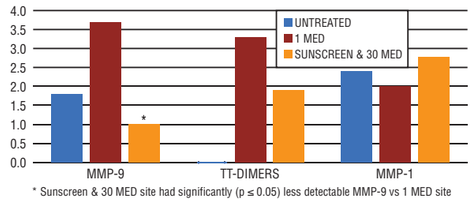
Results

Changes following treatment

Parameter	*Biological Significance*	*Desired Outcome*	*Untreated Control*	*Positive Control (1 MED)*	*Treated Site (30 MED)*	*p-value (1 MED v. 30 MED)*
MMP-9	Collagen degradation	↓	1.82 ± 0.98	3.67 ± 0.58	1.00 ± 0.45	p ≤ 0.05
Mast cells	Inflammation	↓	17.45 ± 6.67	41.20 ± 3.11	25.64 ± 6.10	NSD
Sunburn cells	Cell death	↓	00.0 ± 0.00	2.30 ± 0.73	3.59 ± 3.65	NSD
Thymine dimers	DNA damage	↓	0.00 ± 0.00	3.33 ± 1.15	1.91 ± 0.83	NSD
Vessels	Epidermal damage/Erythema/ Inflammation	↓	19.18 ± 8.70	42.00 ± 8.28	32.55 ± 6.82	NSD
MMP-1	Collagen degradation	↓	2.36 ± 0.50	2.00 ± 0.00	2.73 ± 0.47	NSD
Langerhans cells	Immunoprotection	=	9.39 ± 2.94	6.27 ± 3.79	7.08 ± 2.54	NSD
Vessel dilatation	Erythema formation/Inflammation	↓	0.55 ± 0.52	0.60 ± 0.55	0.64 ± 0.50	NSD

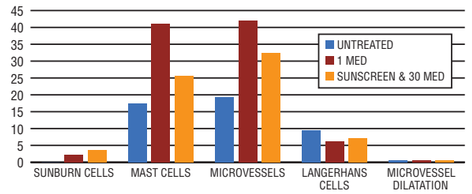
NSD = no significant difference

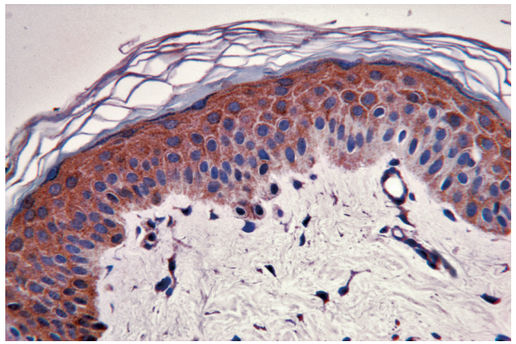
Mean Histological Score (0-4 Scale)



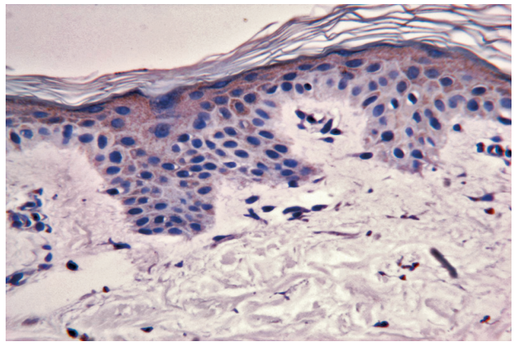
* Sunscreen & 30 MED site had significantly (p ≤ 0.05) less detectable MMP-9 vs 1 MED site

Mean Cell Counts/mm of Epidermis





Mild focal expression within keratinocytes (1+)



Moderate diffuse expression within all keratinocytes (3+)

Note: MMP-9 is a collagenase produced by keratinocytes and dermal fibroblasts in response to certain insults, such as following UV related damage. MMP-9 is most easily assessed histologically, as demonstrated above, via staining of keratinocytes within the epidermis.

Compared with no irradiation, irradiation at 1 MED was associated with an increase in the formation of MMP-9, sunburn cells, mast cells, tt-dimers and microvessels.

The changes in markers of UV-induced cellular damage at the 30 MED irradiated protected site were not significantly different from the unprotected 1 MED irradiated site; in fact, irradiation at 30 MED's generally showed numerically smaller changes in markers of cellular damage than did the positive control.

There was significantly (p ≤ 0.05) less formation of MMP-9, one of the collagen degrading metaloproteinases, at the 30 MED irradiated protected site as compared to MMP-9 formation at the 1 MED site.

Decreases in mast cells and thymine dimers and only a slight increase in sunburn cells, as compared to the positive control, demonstrate the biological protective effect of the test material.

Following irradiation of 1 MED, there were no significant changes in MMP-1, Langerhans cells, or microvessel dilation due to the low energy level in the single exposure. However, no significant changes were seen even after the 30 MED irradiation at the sunscreen treated site, demonstrating the photostable protection.