UV protecting effect of LIPEX® PreAct™

ABSTRACT

The protective effect of Lipex PreAct against UV radiation was studied in human skin explants. The explants were treated with cream formulations on 5 consecutive days and irradiated with 1 or 4 MED UV radiation on Day 6. The explants were analyzed for general morphology and the presence of sunburn cells, thymin dimers and heat shock proteins in histological samples taken on Day 7.

The results show that explants treated with the Lipex PreAct cream show good overall morphology both at 1 and 4 MED radiation. The active cream effectively suppresses the formation of sunburn cells, in contrast to untreated and placebo treated explants. The formation of thymin dimers is decreased with 36% in the samples treated with Lipex PreAct and 1 MED, however, at 4 MED there are no differences between the treatments. Heat-shock proteins (HSP70) in the epidermis are not affected by the treatments, which is explained by the high background levels of HSP70. The results indicate that Lipex PreAct has a protective effect against UV radiation but is not a UV blocker and does not replace traditional organic or mineral UV screens.

INTRODUCTION

Antioxidants in UV protection

Ultraviolet radiation has many adverse effects on the skin, including inflammatory reactions such as erythema, sunburn and, on the longer term, premature skin aging and increased risk for skin cancer. Ultraviolet radiation acts both directly on the skin constituents but also by promoting the formation of free radicals and reactive oxygen species. Protecting exposed skin against UV radiation is therefore seen as a first step to keep a healthy skin over time.

Healthy skin contains several anti-oxidative components, including tocopherols and squalene, as well as enzymes with antioxidative properties. These systems can be depleted on prolonged exposure to sunlight and using a skin care lotion with high levels of antioxidants can to some extent replenish the natural antioxidants.

Lipex PreAct

Lipex PreAct (INCI: Canola oil) is a vegetable oil-based emollient with a high content of natural tocopherols and a fatty acid composition which is stable against oxidation. It is ideally suited for applications where a natural-based stable emollient is required, such as in body lotions and facial care products. The high concentration of Vitamin E makes it a candidate to be used in formulations where an added protection against UV induced skin damage is desired.

Endpoints used in this study

Sunburn cells
Sunburn cells are epidermal apoptotic cells characterized by pycnotic nuclei and a dense orange-colored ectoplasm after staining. They are signs of severe UV induced damage.

Thymine dimers
The formation of thymine dimers is considered to be a sign of DNA damage caused by UV radiation and may lead to mutations if unrepaired. The formation of thymine dimers can be suppressed by the use of antioxidants such as Vitamin C and plant polyphenols.

Heat Shock Proteins
The heat shock proteins are expressed as a response to external stress such as heat and UV radiation. They act by protecting other proteins against conformation changes. They are frequently present in the epidermis and can be induced by external stress in the dermis.

UV protecting effect of LIPEX® PreAct™

STUDY OBJECTIVES

A placebo-controlled study of Lipex PreAct in a simple formulation to study the protective effect against UV radiation. The protective effect is measured by quantifying sunburn cells, thymine dimers and HSP70 (Heat Shock Protein) on histological samples.

EXPERIMENTAL

Sample identification

T - Non-treated (control)
E - Placebo cream
P - Cream with Lipex PreAct

Testing procedure

Explant origin
Skin explants were obtained from an abdoplasty from a 35-year-old Caucasian woman. The explants were 10(+/- 1) mm in diameter and kept in survival.

Sample application
Creams were applied topically at approximately 2 mg/cm2 by spreading the cream on the explant using a spatula. The creams were applied on the explants on Day 0, Day 2, Day 4, Day 5 and Day 6.

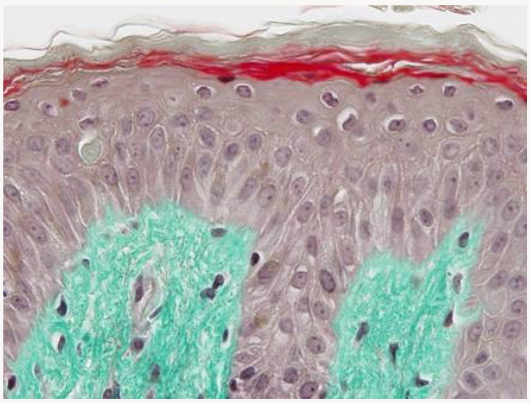
Irradiation schedule
On Day 6, explants were subjected to 1 MED (4.5 J/cm2 UVA and 0.15 J/cm2 UVB) or 4 MED UV radiation (18 J/cm2 UVA and 0.6 J/cm2 UVB).

Analysis of histological samples
Histological samples were prepared on Day 7 and analyzed for general morphology by Masson's trichrome staining, thymine dimers and HSP70 by immunostaining. The amount of sunburn cells (SBC), thymine dimers and HSP70 were quantified from the microscope images and by image analysis.

RESULTS

General morphology, Day 7

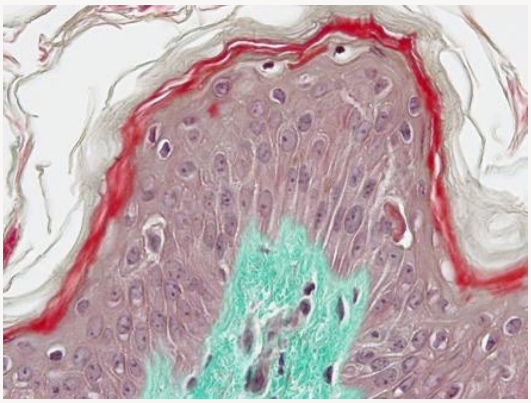
Untreated explants, explants treated with placebo and active cream display normal characteristics after 7 days. The stratum corneum is moderately thick and slightly laminated and keratinized on the surface. The epidermis shows 4 to 5 cellular layers with a good morphology. The relief of the dermal- epidermal junction is rather marked, and the papillary dermis presents a well cellularized structure with moderately thick collagen fibers forming a moderately dense network.



Explant Day 7, non-irradiated

General Morphology, 1 MED

The untreated explants and explants treated with the placebo cream show a rather thick stratum corneum which has a moderately laminated and keratinized surface. The epidermis has 4 to 5 cellular layers with a slightly altered morphology. Some cells with pycnotic nucleus and perinuclear oedema are observed in the epidermis. A small to moderate number of Sunburn Cells (SBC) are also observed.

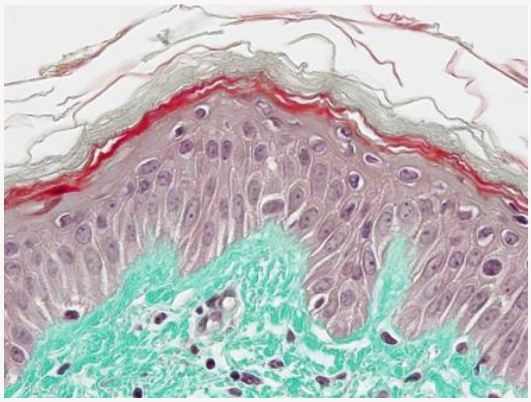


Placebo-treated explant, 1 MED on Day 7

The explants treated with the active cream also have a rather thick, moderately laminated and slightly keratinized stratum corneum. The epidermis shows 4 to 5 cellular layers with a slightly altered morphology.

UV protecting effect of LIPEX® PreAct™

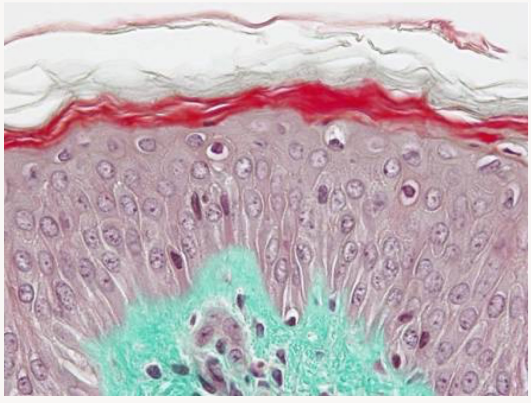
Some cells with pycnotic nucleus and perinuclear oedema and a very small number of Sunburn Cells (SBC) is seen.



Active-treated explant, 1 MED on Day 7

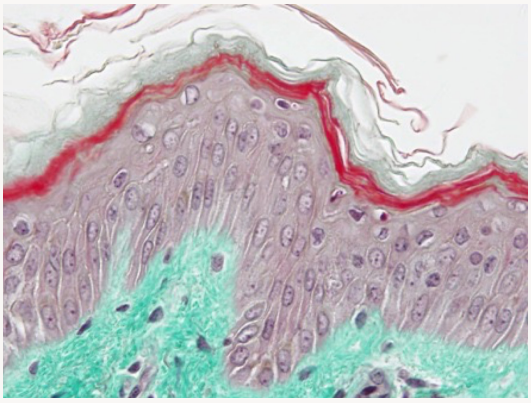
General morphology, 4 MED

The untreated and placebo treated explants have a rather thick, moderately laminated and slightly keratinized stratum corneum. The epidermis has 4 to 5 cellular layers with a moderately altered morphology. Numerous cells with pycnotic nucleus and perinuclear oedema can be seen. A small number of Sunburn Cells (SBC) are also observed, and the dermal cells are slightly altered.



Placebo-treated explant, 4 MED on Day 7

The explants with the active cream show similar characteristics with the exception that sunburn cells are absent and the number of pycnotic cells is markedly lower.



Active-treated explant, 4 MED on Day 7

Sunburn Cells (SBC)

The results from the counting of sunburn cells are shown in Table 1. Irradiation with 1 or 4 MED causes the formation of SBC in unprotected explants. The placebo cream has a shielding effect at 1 MED resulting in fewer SBC compared to the untreated explant. Explants treated with the active cream show good protection and a total absence of SBC.

Summary of sunburn cell count (SBC/cm)

	NI		UV1		UV2	
Treatment	Mean	SD	Mean	SD	Mean	SD
T	0	0	5.6	3.6	1.5	0.9
E	0.3	0.6	0.3	0.5	0.6	0.5
P	0	0	0	0	0	0

Thymine dimers

The non-irradiated explants showed a small amount of thymine dimers on Day 7, regardless of any treatment with creams (expressed as the surface percentage in the living epidermis, Table 2). The surface coverage in the placebo treated and untreated samples was about 10-12% while the active cream showed a significantly lower value of 7%.

When irradiated with 4 MED, all treated explants showed similar values of 28-30%.

UV protecting effect of LIPEX® PreAct™

Summary of thymine dimer quantification (area-%)

	NI		UV1		UV2	
Treatment	Mean	SD	Mean	SD	Mean	SD
T	0.2	0.2	10.3	2.7	27.5	4.9
E	0	0	12	3.6	29.6	5.1
P	0	0	6.6	3.1	28.2	4.1

HSP 70

The amount of HSP 70 is high in all samples (Table 3), regardless of irradiation. There is no consistent pattern in the evolution of the HSP 70 levels. See discussion for possible explanations.

Summary of HSP70 quantification (area-%)

	NI		UV1		UV2	
Treatment	Mean	SD	Mean	SD	Mean	SD
T	56.4	15.6	73.2	15.3	59.6	13.9
E	81.1	11.7	40.4	18.4	58.1	17.4
P	55.6	17.7	67.3	13.3	66.5	16.5

DISCUSSION

General morphology

The general morphology of the non-irradiated explants after 7 days is good, with no changes induced by the treatment. UV irradiation with 1 MED induces slight alterations to the morphology in the non-treated and placebo treated explants with appearance of sunburn cells. The explants treated with the active cream containing Lipex PreAct show good overall morphology at both 1 and 4 MED

Sunburn cells

Sunburn cells are observed in the non-treated explants at both 1 and 4 MED, with the number increasing at the higher irradiation dose. The placebo formulation offers some protection at 1 MED but not at 4 MED, indicating that there is a shielding effect from the formulation itself. The active cream with Lipex PreAct effectively suppresses the formation of sunburn cells, both at 1 and 4 MED.

Thymin dimers

Thymine dimers, as expected, are not found in the non-irradiated explants at Day 7. UV irradiation with 1 MED increases the area occupied by thymine dimers to about 10-12 % in the non-treated and the placebo-treated explants, showing that the placebo cream is not protecting against thymine dimer formation. On the other hand, the active cream with Lipex

PreAct shows only 6.6 area-% occupied by the thymine dimers, corresponding to about 36% protection. With 4 MED no protective effect is seen by the formulations and the surface area occupied by thymine dimers is about 28-30%.

HSP 70

The level of HSP 70 in the epidermis is usually high as the outer part of the skin is constantly exposed to environmental stress (Roh et al, 2008). In the present study the levels of HSP 70 are high already in the untreated explants and the levels are not consistently affected by the treatments. The standard deviation on the measured data is also high. It is therefore not possible to draw any conclusions from the HSP 70 measurements. It would have been interesting to see if there is an effect on the dermal fibroblasts but this aspect was not studied.

CONCLUSION

The results of the study show that Lipex PreAct can help to protect the skin against the harmful effects of UV radiation. Damage markers such as sunburn cells and thymine dimers are suppressed under the experimental conditions. It is important to note that Lipex PreAct does not replace traditional organic and inorganic UV screens.

REFERENCES

Roh BH, Kim DH, Cho MK, Park YL, Whang KU. Expression of Heat Shock Protein 70 in Human Skin Cells as a Photoprotective Function after UV Exposure. Ann Dermatol. 2008 Dec;20(4):184-9. doi: 10.5021/ad.2008.20.4.184. Epub 2008 Dec 31. PMID: 27303188; PMCID: PMC4903975.

Access this article online

hub.aakpersonalcare.com/uv-protecting-effect-of-lipex-preact