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Bioactive Sphingolipids for DNA protection

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Introduction:

Sunlight triggers deleterious oxidative stress in the skin. The generation of reactive oxygen species (ROS) is a common process occurring during various cellular reactions. An overproduction or inadequate processing of ROS within the skin manifests itself on a biomolecular level by lipid peroxidation, protein degradation, enzyme dysfunction and even DNA mutations/breakage. Sphingolipids are well known for their contribution and relevance for a proper skin barrier function. Therefore, innovative bioactive Hydroxy-Ceramides were developed and screened for their biological activity.

Innovative bioactive sphingolipid Hydroxybutyroyl Phytosphingosine

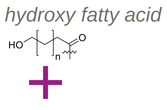
PRODUCT PROPERTIES

INCI (proposed)	Hydroxybutyroyl Phytosphingosine
Use level	0.02 - 0.2% (clinically tested at 0.1%)
Product form	pure powder (100% active matter)

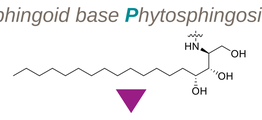
COMPOSITION
Composed of a sphingoid base and an amide-bond omega hydroxy fatty acid with short chain length.

PROPERTIES
Due to the short chain fatty acid and hydroxy groups, the molecule can pass through the skin barrier to provide bioactive functions in the deeper epidermidis layers and dermis.

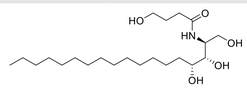
Omega hydroxy fatty acid



Sphingoid base Phytosphingosine



Hydroxybutyroyl Phytosphingosine



Materials & Methods:

In vitro studies

SimDerma® Screening
SimDerma® is a screening system that includes 23 laboratory assays. This tool has been developed to identify novel biological activities for cosmetic and skincare products. Hydroxybutyroyl-Phytosphingosine was screened using SimDerma® to offer a wide and fast overview of the ingredient's activity profile and potential skin care claims.

Protection from environmental influences (keratinocytes)

- Normal human epidermal keratinocytes (NHEK)
- Treatment with test substances for 24h, UV-irradiation [250 mJ/cm² UVB +1.6 J/cm² UVA]
- Reactive oxygen species (ROS) DNA damage (comet assay)

Protection from environmental influences (epidermal skin models)

- Reconstructed human epidermal skin models
- Systemic treatment with test substance for 24h, UV-irradiation [500 mJ/cm² UVB +3.2 J/cm² UVA]
- Sunburn cells (SBC) via HE staining Cyclobutene pyrimidine dimers (CPD)

In vivo study

Regeneration of sun-stressed skin

- 16 panelists per test formulation Sun-stressed skin (after summer period)
- O/W cream with 0.1% Hydroxybutyroyl-Phytosphingosine, Vehicle Application twice daily on forearm
- Start 2 weeks, 4 weeks, 8 weeks

Skin tone
The skin tone was evaluated on the outer forearm measuring skin color parameters with a colorimeter (L* and ITA: the higher the value, the lighter the skin color).

Skin texture
Skin texture was measured on the inner forearm using a Visioscan VC 98 camera. Various texture parameters were summarized to show an overall skin texture value (calculated as % improvement of the initial value).

Skin structure
Skin structure was evaluated by measuring skin roughness (Visioscan VC 98, summarized roughness values) and skin density (Ultrasound, Dermascan C, Cortex Technology, Denmark) on the outer forearm.

Results & Discussion:

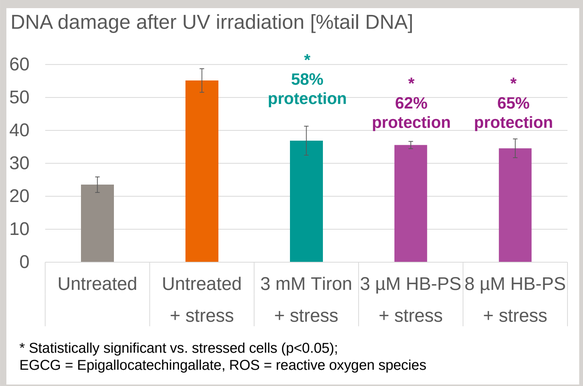
SimDerma® screening as first step in product development shows the main activity profile for Hydroxybutyroyl Phytosphingosine in "Skin Defense".

- ROS inhibition
- CB1 Antagonism
- STAT3 inhibition

Experimental models	Age-Defying			Sensitive Skin		Nourishing		Skin Evenness		Skin Defense				Barrier Fortify			Hair Care
ROS Inhibition (k)	+++	+++	·	·	+++	+++	·	+++	+++	+++	+++	+++	+++	+++	+++	·	+++
CB1 Antagonism	·	·	·	·	·	·	·	·	·	+++	·	·	·	·	·	·	+++
STAT3 Inhibition	·	·	·	·	·	·	·	·	·	+	·	+	·	·	·	+	+

+ low activity, ++ medium activity, +++ high activity, · No relation
(f) fibroblasts, (k) keratinocytes, (m) melanocytes, (mac) macrophages

Protection from UV induced DNA damages – comet assay

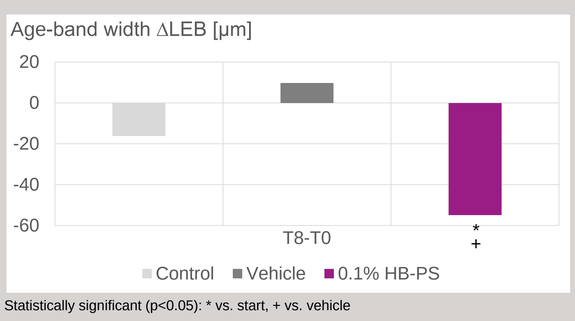


* Statistically significant vs. stressed cells (p<0.05);
EGCG = Epigallocatechingallate, ROS = reactive oxygen species

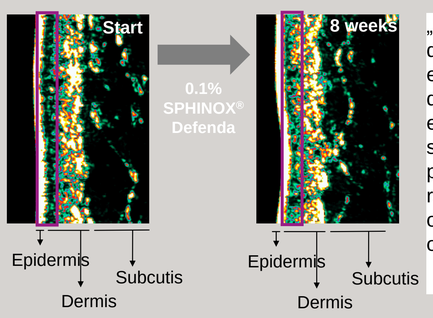
Hydroxybutyrol Phytosphingosine (HB-PS)

- reduces oxidative cell stress
- protects against UV induced DNA damage

Reduction of UV-induced age-band formation – *in vivo* study



Statistically significant (p<0.05): * vs. start, + vs. vehicle



„Age-bands" can develop between epidermis and dermis after sun-exposure as a sign of photoaging. They reflect a reduction of skin density by oxidative stress.

Hydroxybutyroyl Phytosphingosine (HB-PS)

- reduces the width of age-bands
- improves skin structure of sun-damaged skin
- can protect from sun-induced premature aging

Conclusions:

Hydroxybutyroyl Phytosphingosine showed promising anti-oxidative benefits in a broad screening approach (SimDerma®) with a special efficacy in DNA protection. Positive effects on sun-stressed skin could be shown *in vivo*. A re-balanced skin tone after summer stress might be due to an accelerated skin regeneration based on DNA protection. Overall, a protection from sun-induced premature aging could be shown which marks Hydroxybutyroyl-Phytosphingosine as a multifunctional product for holistic skin protection. Since DNA protection efficacy has not yet been described for Sphingolipids, further studies were initiated to investigate the exact working mechanism. In UV-exposed epidermal skin models it was observed that Hydroxybutyroyl Phytosphingosine has a significant impact on epigenetic biomarkers. Preliminary results indicate that Hydroxybutyroyl Phytosphingosine impacts at least two histone post-translational modifications H3K9Me3 and H3K18Ac (unpublished results). H3K9Me3 is linked to photo-aging, while H3K18Ac is described in the context of DNA repair. These findings will be consolidated in the next step.

Acknowledgements:

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