Sphingolipid promotes multilayer skin communication

ABSTRACT

Communication between the cells within a particular skin layer, but also across different skin layers and between different skin cell types is very important for the proper function of the skin. The unique short-chain ceramide, Sphingokine® NP, was found to stimulate the cross-talk between cells throughout the skin. It provides multilayer activity improving the state of the various skin layers. It functions as a signalling sphingolipid. Due to deep penetration of Sphingokine NP, the molecule can reach all different skin layers from the epidermis to the dermis and even the subcutaneous tissue.

The skin, as the largest human organ, functions as a mechanical and chemical protection barrier between the body and the environment. It is made up of different layers, the outermost epidermis with the main barrier function and the subjacent dermis. Below the dermis the hypodermis or subcutaneous tissue is located.

The epidermis is the outermost layer of the skin. A properly built and structured epidermis is necessary to protect the skin from microorganisms, dehydration, mechanical stress and environmental influences.

The main cell types present in the epidermis are keratinocytes. The keratinocytes in the basal layer of the epidermis (stratum basale) take a journey to the skin surface undergoing a well-defined characteristic terminal differentiation. During keratinocyte differentiation the cells accumulate large amounts of lipids which they deposit to create the lipid lamellae ('mortar') of the stratum corneum. The cemeocytes, together with the surrounding lipids, form the protection barrier of the skin.

The dermis is located just beneath the epidermis and is very important for the skin's strength and resilience. This skin layer displays a relatively small number of cells and mainly consists of connective tissue. Fibroblasts, as the major cell type in the dermis, are primarily responsible for production of dermal extracellular matrix (ECM) which determines the properties of the connective tissue. The ECM can serve many functions, such as providing support and structure for the skin, regulating cells' dynamic behaviour, and regulating intercellular communication. The dermis is tightly connected to the epidermis through a basement membrane (also called dermal-epidermal junction) which is the communication channel between the epidermis and dermis. The dermis supports the epidermis by providing it with nutrients.

The main structural components of the dermis (ECM) are collagen, elastic fibres (elastin, fibrillin) and extracellular matrix (glycosaminoglycans like hyaluronic acid).

The subcutaneous adipose tissue is located just beneath the dermis, and is composed of lipid-filled cells called adipocytes. Until recently, adipocytes were considered only as an inert fat-storing tissue. However, latest studies have demonstrated that adipocytes play dynamic roles in highly regulated processes leading to the assumption that there is a substantial interaction between

Figure 1: Loricrin, involucrin, and transglutaminase 1 gene expression in human primary keratinocytes after treatment with Sphingokine NP for 72 hours. The results are normalised to β-Actin (ACTB) gene expression as reference gene, and calculated relative to vehicle.
subcutaneous adipose tissue and the dermal skin layer. Adipocytes secrete various factors like adiponectin and leptin that influence other tissues. It could be shown that adiponectin and leptin increase the production of both hyaluronic acid and collagen in dermal fibroblasts. That means that the adipose tissue plays an important role in dermal elasticity.

Communication between the cells within a particular skin layer, but also across different skin layers and between different skin cell types is very important for the proper function of the skin. This communication takes place via cell signalling molecules like adiponectin and leptin which are capable of controlling the action of the surrounding tissue.

The unique short-chain ceramide, Sphingosine NP, was found to stimulate the cross-talk between cells throughout the skin. Sphingosine NP is based on fermentative production from biorenewables. The phyto-sphingosine backbone of this short-chain ceramide possesses skin identical stereochemistry due to a patented biotechnological production process.

In vitro tests
In a large screening study, Paragh et al. have demonstrated that short-chain ceramides, like caprooyl phytosphingosine (Sphingosine NP), significantly stimulate skin cell differentiation in cell culture. Now the effect of caprooyl phytosphingosine in human primary keratinocytes was analysed in order to determine its influence on the formation of the skin barrier.

Results
A gene expression analysis in human differentiating epidermal keratinocytes was performed. During this study it was observed that caprooyl phytosphingosine triggers expression of the late-stage epidermal differentiation marker loricrin up to 3-fold in human primary keratinocytes after 72 hours of stimulation (Fig. 1). Similar results have also been observed for other differentiation markers, i.e. involucrin and transglutaminase.

The result of this study demonstrates that in the upper layers of the epidermis (mimicked here by using differentiated keratinocytes) caprooyl phytosphingosine stimulates the generation of the cornified epidermal barrier and renewal of the epidermis by stimulating keratinocyte differentiation. This leads to strengthening, densifying and smoothing effects of the stratum corneum, the protection barrier of human skin.

Additional DNA chip studies on human proliferating epidermal keratinocytes as well as dermal fibroblasts and adipocytes were conducted. The results show that in the deeper layers of the viable epidermis caprooyl phytosphingosine might be able to improve the cross-talk between keratinocytes (epidermis) and fibroblasts (dermis). Thereby, caprooyl phytosphingosine supports the dermal ECM resulting in tighter skin. On fibroblasts, caprooyl phytosphingosine improves the dermal extracellular matrix organisation and structure and supports the dermal scaffold function. This leads to a reshaped and tighter dermis which strengthens the skin structure. The results of the study on adipocytes show that caprooyl phytosphingosine highly stimulates human adipocytes. The genes that are regulated indicate that caprooyl phytosphingosine improves the cross-talk between adipocytes and fibroblasts, leading to support of dermal extracellular matrix formation. In addition, the adipose matrix will be strengthened and lipogenesis will be modified leading to more dense and plumped adipose tissue by caprooyl phytosphingosine. The gene expression results on adipocytes have further been substantiated by additional protein analysis.

Figure 2: Degree of sagging skin relative to the beginning after 4, 8, and 12 weeks of application (compared to vehicle) of Sphingosine NP graded by experts.

Figure 3: Digital images of two representative volunteers before, and after, application of 0.2% Sphingosine NP.
**ANTI-AGEING**

**In vivo study**

Based on the in vitro results, a facial in vivo study on Caucasian women (aged 50-70 years) was performed. The aim of this study was to evaluate the efficacy of caprooyl phytosphingosine in improving the signs of gravitational ageing like sagging skin. Both formulations, one containing 0.2% caprooyl phytosphingosine and the vehicle formulation were each tested by 30 volunteers in a half-side test. The formulations were applied twice daily on the face. The skin measurements were carried out at the beginning and after 4, 8, and 12 weeks in temperature and humidity-controlled rooms. The following parameters have been evaluated: the sagging degree of the skin, skin roughness (wrinkle depth), skin density/echogenicity (not shown here). Finally, digital images have been taken.

**Results**

Sagging skin was evaluated by expert grading. Figure 2 shows the degree of skin sagging during the application period. Treatment with caprooyl phytosphingosine decreased skin sagging after 8 weeks and maintains the results through extended application. The results were statistically significant compared to the start value.

The reduction of sagging skin degree after application of caprooyl phytosphingosine can also be seen in the images in Figure 3.

The sagging skin regions in the chin area have been improved and the contour of the face is visibly lifted. In addition, it can be seen that the depth of oral commissures and smile lines in the area at the mouth can be reduced by caprooyl phytosphingosine.

In addition to the improvement of sagging, it could be shown that application of caprooyl phytosphingosine led to a decrease of skin roughness. This effect was statistically significant compared to the vehicle control after 8 weeks of application. After 12 weeks an approximately 5-fold improvement of wrinkle depth compared to the vehicle formulation could be observed (Fig. 4).

These effects can also be seen in the PRIMOS Pico images in Figure 5. The depth of the wrinkles near the eye is visibly reduced 12 weeks after application of 0.2% caprooyl phytosphingosine.

**Conclusion**

The results of these studies demonstrate that Sphingokine NP reduces signs of gravitational ageing. Sphingokine NP has a multilayer activity which could be shown in various in vitro studies on different skin cells. It improves keratinocyte-fibroblast and adipocyte-fibroblast cross-talk. Thereby, Sphingokine NP provides a reshaped dermal scaffold and supports the structure of adipose tissue leading to plumped and densified skin. These effects lead to reduced skin sagging, significantly tighter skin and toned skin tissues.

In addition, as shown by the in vivo study, wrinkles can be flattened by application of Sphingokine NP.

Taken together, Sphingokine NP is an innovative active ingredient for different kinds of anti-ageing applications like anti-sagging products, shaping creams for face and body and products for improving facial contours.

**References**


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**Figure 4:** Skin surface roughness (Sa)/wrinkle depth relative to the beginning after 4, 8, and 12 weeks of application of Sphingokine NP (compared to vehicle).

**Figure 5:** PRIMOS Pico images of two representative volunteers, before, and after application of 0.2% Sphingokine NP.