Preventing hair loss by balancing the hair cycle, strengthening the hair follicle, and improving scalp health

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Abstract

The overall impression that people make depends to a significant degree on the appearance of their hair. Aging and conditions such as androgenic alopecia result in hair follicle miniaturization, which shortens the growth phase, resulting in hair loss and the formation of fine and lifeless hair.

Healthy hair embedded in a healthy scalp is the key to preventing hair loss.

Scientifically well-founded claims have been made that substantiate the action of Sphinganine, a skin-identical sphingolipid that targets hair loss by balancing the hair cycle, strengthening the hair follicle, and improving scalp health.

Hair growth occurs over a cycle consisting of growth (anagen), regression (catagen), resting (telogen), and exogen phases\(^1, 2\) (figure 1).

Sphinganine-based ceramides represent the most abundant ceramide class present in human hair\(^3\). Based on this and other scientific data, sphinganine seems to be a promising active ingredient for hair-quality promoting cosmetic applications.

Sphinganine is produced from renewable raw materials such as sugar in a sustainable biotechnology process.

The fermentative production process ensures that Sphinganine features the same stereochemical configuration as found in nature and in human skin (figure 2). The skin-identical stereochemistry of Sphinganine is of key importance for its biological functions.

Figure 1: The hair growth cycle

Sphinganine prolongs the anagen phase and improves scalp health and scalp renewal capacity, making it an ideal active ingredient for preventing hair loss (figure 3a, 3b).

Figure 2: The chemical structure of Sphinganine. The absolute stereochemistry on the asymmetrical carbon atoms is indicated in brackets (in purple)

Sphinganine promotes hair growth by prolonging the anagen phase

Figure 3a: Proposed working mechanism of Sphinganine within the hair life cycle

Sphinganine improves scalp health by equalizing the micro flora

Figure 3b: Proposed working mechanism of Sphinganine within the hair follicle and on the scalp

Figure 3: Proposed working mechanism of Sphinganine within

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Various *in vitro* studies have shown that Sphinganine balances the hair cycle by inhibiting 5-α-reductase, by improving the capacity of the scalp to renew itself, by stimulating the formation of essential building blocks such as proteins and ceramides, and by balancing microflora to improve scalp health.

**Preventing hair loss by inhibiting 5-α-reductase (*in vitro*)**

The aim of this study was to determine the potential of Sphinganine as an inhibitor of 5-α-reductase type I, which is the key enzyme catalyzing irreversible reduction of testosterone (T) to dihydrotestosterone (DHT), the most potent androgen. DHT has been identified as key factor involved in androgenetic male baldness.

The inhibitory potential towards 5-α-reductase type I was tested in a cell-free assay using cell homogenates isolated from stably transfected HEK293 cells, yielding an IC₅₀ of 6.6 µM for Sphinganine (figure 4). Finasteride, a known 5-α-reductase inhibitor, was included as the internal positive control.

The test results show that Sphinganine is a potent inhibitor of 5-α-reductase type I. Consequently, Sphinganine is a suitable cosmetic ingredient for inhibiting the conversion of testosterone to dihydrotestosterone. This will positively influence the hair growth cycle by preventing the transition to the catagen phase and follicle miniaturization, particularly among men.

Moreover, reduction of scalp sebum production will lead to a less greasy scalp and an overall improvement in scalp health.

**Improvement of the scalp barrier function and renewal capacity (*in vitro*)**

A functional skin barrier is a prerequisite for a healthy scalp. By contrast, compromised barrier function can lead to a dry, itchy scalp. Improving the barrier formation should therefore lead to an improvement in overall scalp health, with the additional, indirect benefit of combatting hair loss.

The aim of the study was therefore to determine the global effects of Sphinganine treatment in a confluence-induced, *in vitro* keratinocyte differentiation model, particularly with respect to lipidomic and transcriptomic changes.

Sphinganine treatment strongly promotes ceramide production in keratinocytes (figure 5).

Overall ceramide pools were stimulated in a balanced way, leading to concomitant upregulation of all major ceramide classes containing sphinganine, phytosphingosine, or sphingosine in their backbones. In particular, very long-chain fatty acid ceramides, which are deemed critical for intact barrier function, were upregulated. Moreover, microarray analysis (data not shown) indicates significant induction of overall sphingolipid metabolism and keratinocyte differentiation caused by the Sphinganine treatment.

In addition to applying Sphinganine on differentiating epidermal keratinocytes, a complementary experiment was conducted on a non-differentiating keratinocyte model in order to explore effects on the transcriptome of proliferating epidermal keratinocytes. The purpose of this experiment was to explore the effects of Sphinganine once it had penetrated deeper layers of the viable epidermis, the follicle shaft, and the hair root. Because the hair shaft arises from rapidly proliferating keratinocytes in the bulb (one of the highest rates of proliferation in the body), special attention was paid to genes known for their roles in cell proliferation. This class of genes includes growth factors, as well as genes controlling synthesis and turnover of the intracellular messenger molecule sphingosine-1-phosphate. Another class of genes relevant in the context of proper hair structure is one that mediates cell-cell interaction and includes integrin and cadherin genes.

In summary, the gene expression data obtained for undifferentiated, proliferating keratinocytes suggest that Sphinganine maintains the proliferative state of keratinocytes and favors epidermal renewal (figure 6).
In particular, the stimulation of basic fibroblast growth factor expression, as well as the stimulation of gene expression related to sphingosine-1-phosphate and sphinganine-1-phosphate synthesis, indicates that treating keratinocytes with Sphinganine triggers signaling cascades that ultimately promote cell proliferation and counteract apoptosis. Assuming that these signaling cascades are conserved, it seems likely that the positive effects of Sphinganine treatment also apply to hair matrix keratinocytes, which represent one of the fastest proliferating cell populations in the body. As such, Sphinganine will likely keep hair follicles in an active, growing state, and prevent follicles from prematurely undergoing apoptosis and entering the catagen phase. Moreover, developing hair follicles benefit from inducing genes for cadherin and integrin, two classes of proteins that mediate cell-cell and cell-matrix adhesion processes, and that are critically important for proper hair follicle and hair shaft architecture.

**Improvement in scalp health by equalizing microflora (in vitro)**

Overall scalp health is always the outcome of multiple influencing factors. One important factor is scalp microflora. While the natural scalp microflora comprises many different microbe species, yeast strains belonging to the genus *Malassezia* have been identified as a major determinant of scalp health. Numerous studies have demonstrated the key role that *Malassezia* strains play in the context of scalp health, particularly with respect to dandruff and seborrheic dermatitis. Although *Malassezia* strains are part of normal cutaneous microflora, they are known to be involved in the occurrence and severity of certain human skin conditions, including dandruff, seborrheic dermatitis, folliculitis, and others.

Another mechanism contributing to a healthy scalp is mediated via the expression of anti-microbial peptides (AMPs). These peptides act mainly by interfering with the cytoplasmic membrane of target microbe strains, causing membrane leakage and ultimately cell death. One of the most prominent anti-microbial peptides is human β-defensin 2 (HBD2). Expression of AMPs correlates with the keratinocytes' differentiation status, and together with the mechanical stability conferred by the stratum corneum, AMPs efficiently help maintain overall skin and scalp health.

Sphinganine improves scalp health directly via antimicrobial activity and indirectly by stimulating HBD2 formation.

An *in vitro* germ count reduction test revealed profound direct anti-microbial activity against *Malassezia furfur* (figure 7), which is known to cause dandruff. Moreover, Sphinganine treatment stimulates HBD2 gene expression in an *in vitro* keratinocyte model (figure 8).

**Extended in vivo studies have confirmed that Sphinganine significantly reduces hair loss by prolonging the anagen growth phase of hair and effectively improves hair quality and scalp health.**
The TrichoScan® method was employed to objectively determine state of the hair life cycle by determining the anagen and telogen rate. Visible and otherwise detectable effects were assessed by expert rating and photographic documentation.

An ethanolic hair tonic containing 0.1 - 0.5% Sphinganine was provided, and volunteers evenly applied this to their scalps (dry hair) and hairlines in the morning and evening, aided by some gentle massage. Measurements were conducted before the first application and after 8 and 16 weeks of product application. Data were considered from a total of 96 test subjects.

The TrichoScan® images clearly show the effects of Sphinganine application (figure 9). The increase in anagen hair leads to a noticeable increase in the amount of hair on the scalp. The TrichoScan® images also indicated beneficial effects on scalp health.

Groups, the anagen rate increased, and the increase was highest in the 0.5% Sphinganine group (2.7% and 2.8% after 8 and 16 weeks, respectively). In the 0.2% and 0.1% Sphinganine groups, increases of 1.8%/1.9% and 1.1%/1.1% were determined (after 8/16 weeks, respectively).

Relative to vehicle control, the increase in the anagen rate in the 0.5% Sphinganine group was 4.3%/3.9% (after 8 and 16 weeks, respectively), 3.3%/3.0% in the 0.2% Sphinganine group and 2.7%/2.3% in the 0.1% Sphinganine group.

In terms of the expert rating (figure 12), all hair quality and scalp health parameters improved after application of Sphinganine, regardless of the concentration. In particular, hair quality parameters were greatly improved in all Sphinganine groups compared to vehicle control, and improvements in hair volume scored best.
Conclusion

Overall, SPHINGONY (trade name for Sphinganine) is a naturally occurring, skin-identical molecule particularly targeting male pattern hair loss by balancing the hair cycle, strengthening the hair follicle, and improving scalp health. SPHINGONY is COSMOS certified and approved by Ecocert Greenlife according to the Ecocert Standard for Natural and Organic Cosmetics, available at http://cosmetics.ecocert.com.

References

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