Phytosphingosine: A Nature-inspired Sphingoid Base With Multiple Skin Benefits

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ABSTRACT: Phytosphingosine is an important component of ceramides that also exists as a free base in small quantities in the stratum corneum. Recently manufactured biotechnologically, it can act as an antimicrobial, anti-inflammatory, epidermal pro-differentiation mediator and an anti-acne compound, as this literature review shows. Through these activities, phytosphingosine is suggested as a skin-identical approach to skin care.

Within the market demand for natural solutions, consumers seek products with skin-identical approaches to maintain and improve skin health and beauty. In response, formulators can develop products to improve skin function by focusing on activity in the surface layers of skin as well as stimulating activity from within. In this context, ceramides, which are present in skin and consist of N-acylated sphingoid bases, are well-established in the literature for their importance in skin barrier function and stratum corneum (SC) moisturization.

However, less has been published on the effects of free sphingoid bases that are also present in skin. Phytosphingosine, typically an 18-carbon chain that incorporates a 2-amino-1,3,4-triol for its lipid head group, is a free sphingoid base that constitutes part of the chemical antimicrobial barrier of the SC to help control infection. In addition, it can serve many other activities, which are the subject of this literature review.

Phytosphingosine as a PPAR Ligand

The anti-inflammatory and pro-differentiating benefits of phytosphingosine, which will be discussed later in this article, occur through its action as a peroxisomal proliferator activated receptor (PPAR) ligand. Nuclear hormone receptors exert their effects directly on genes by binding to DNA upon their activation by a receptor ligand. As an example, readers are likely familiar with the effects of retinoic acid and its mediation via the retinoid receptors, which are part of the superfamily of nuclear hormone receptors. A coordinate control of two families of transcription factors exists that mediates retinoic acid’s effects, namely the retinoid-X-receptor (RXR) and the retinoic acid receptor (RAR). The active transcription factor complex is formed by heterodimerization of the two receptors and binding of their retinoic acid metabolites.

PPARs are also members of this family and have been shown to be important in the regulation and catabolism of dietary fats, stimulation of epidermal differentiation, reduction of inflammation, and reduction of melanocyte proliferation and tyrosinase levels, together with reducing the signs of aging. Like the retinoid receptor complex, PPARs also form an active complex with RXR; this suggests that the combination of phytosphingosine and retinol might be advantageous since retinoid binding would activate the RXR receptor while phytosphingosine binding would activate the PPAR receptor. The dimerization of both activated receptor molecules would thus trigger highly specific molecular processes related to the above-mentioned effects.

Through its action as a PPAR ligand, phytosphingosine exhibits anti-inflammatory and pro-differentiation effects.

These activities are regulated through one or more of the three isoforms of PPARs: PPARα, PPARδ and PPARγ. In human skin, epidermal differentiation is predominantly regulated by PPARδ and, to a lesser extent, by PPARα; inflammation through PPARα and some PPARγ; and melanocyte proliferation mainly via PPARγ. Fibroblast function is believed to be mediate via PPARα. Downie et al. used human chest sebaceous glands as 7-day cultured whole organs and were able to demonstrate that activators of PPARα and PPARγ inhibited the rate of sebaceous
lipogenesis and reduced the synthesis of the sebum specific lipids squalane and triacylglycerol in human sebaceous glands. They concluded that since the suppression of sebum secretion is associated with reduced acne activity, the nuclear hormone receptors involved may open new avenues in the development of novel acne treatments. Most recently, Ottaviani et al. reported that increased expression of PPARs induced by squalene peroxides in HaCat keratinocytes leads to the down-regulation of inflammatory mediators.

In vitro, the influence of PPAR agonists on sebocyte lipogenesis is controversial. The work of Zoumboulis suggests that PPAR agonists stimulate sebaceous lipogenesis. Recently, however, PPARγ agonists rather than antagonists have been reported to decrease sebaceous gland size and the dihydro testosterone effects on the glands in the Fuzzy rat. Since the consensus of the effects of PPAR ligands was considered positive, the authors were interested in the effects of sphingoid derivatives as PPAR agonists. In this respect, Van Veldhoven et al. first identified that sphingoid bases interact with PPARγ, particularly sphingenine, sphinganine and 4D-hydroxysphinganine. Although they reported that N-acyl sphingoids (ceramides) did not bind, Tsuji et al. found that certain synthetic ceramide analogues bound to all three PPAR isoforms.

On the other hand, Tan et al. reported that ceramides are PPAR agonists. More importantly for the present discussion, Kim et al. demonstrated that phytosphingosine activated the transcriptional activity of PPARs in reporter gene assays. Equally, phytosphingosine was shown to be a pan PPAR ligand in the order PPARγ, PPARα and PPARδ with 57%, 36% and 17% activity (see Figure 1). This suggests that phytosphingosine can activate the whole PPAR system and may have multiple skin benefits.

Moreover, in the same study, real-time polymerase chain reaction (R-T PCR) analysis demonstrated that the mRNA of PPARγ was increased in a dose- and time-dependent manner. A maximum 3.8-fold induction was obtained with a 5 μM concentration of phytosphingosine after a 24-hr treatment. In addition, the authors recently demonstrated a 1.5-fold induction of PPARδ in primary human keratinocytes (data unpublished). The effects of phytosphingosine acting as a PPAR agonist as well as its ability to stimulate the induction of its cognate receptor suggest it would impart pleiotropic skin benefits.

**Figure 1. The effects of phytosphingosine on the transcriptional activity of PPARs (+); the relevant PPAR isoform is transected into HaCat cells. Note: Data represents mean ± SD; *significant at p < 0.05; **significant at p < 0.01**

**Figure 2. The effects of phytosphingosine on the formation of key factors involved in SC formation; relative expression of involucrin (gray bars), transglutaminase-1 (dark gray bars) and filaggrin (black bars) in keratinocytes after stimulation with 5 μM phytosphingosine, compared with untreated time-matched controls. Loricrin is shown on a different scale in the inset graph (gray hashed bars).**

**Phytosphingosine in Keratinocyte Differentiation**

Considering the described mechanisms, the authors first explored literature on the role of phytosphingosine in epidermal differentiation, since this function is impaired in aged and dry skin. With this condition, aberrations in the formation of the SC and corneocytes occur and in this setting, involucrin and loricrin are important for the structural integrity of the corneocytes of the corneocyte envelope. In the case of
involucrin, the attachment of covalently bound lipids, i.e. ceramides and fatty acids, mediated by transglutaminase-1, is essential for it to act as a scaffold to assist in the subsequent formation of the lamellar bilayer structure of the SC intercellular lipids. Equally, filaggrin, the precursor to skin’s natural moisturizing factor, also is involved in the compaction and flattening of keratinocytes to corneocytes. Grether-Beck et al. demonstrated that phytosphingosine significantly induced the expression of four key keratinocyte differentiation proteins; involucrin by 7-fold, loricin by 150-fold, transglutaminase-1 by 32-fold, and filaggrin by 37-fold (see Figure 2).

Paragh et al. examined the prodifferentiating effects of phytosphingosine by computational identification of transcription factor binding sites in the gene promoters of a variety of epidermal differentiation-specific transcription factors. Composite promoter models were generated to explain these effects; interestingly, the activator protein-1 protein complex was included in these models (jun and fos proteins). The average gene expression of 53 keratinocyte differentiation marker genes was calculated and phytosphingosine was shown to stimulate them significantly, similar to the effects of vitamin D3.

**Phytosphingosine as an Anti-inflammatory**

As discussed previously PPARα and PPARγ agonists have been shown to reduce cutaneous inflammatory reactions; however, phytosphingosine also was shown to inhibit irritant contact dermatitis produced by the topical application of 12-0-tetradecanoylphorbol-13-acetate (TPA). It also inhibited epidermal thickening and edema associated with the infiltration of inflammatory cells into the skin by blocking the TPA-induced generation of the pro-inflammatory mediator prostaglandin E2.

Pavicic et al. examined the release of interleukin-1α by untreated, UVB-irradiated human skin explants and found that IL-1α release was increased by a factor of 4.2, compared with non-irradiated skin. However, topical treatment with 0.2% phytosphingosine markedly inhibited the release of IL-1α by 78%, compared with untreated UVB-exposed skin.

To explain these results mechanistically, the effects of phytosphingosine on protein kinase C (PKC), an enzyme involved in downstream signaling in inflammation, were also examined. At concentrations of 0.01%, 0.1% and 0.2%, phytosphingosine inhibited approximately 90% of PKC activity compared with the untreated control. Similarly, phytosphingosine was found to inhibit the inflammatory effects of sodium dodecyl sulphate (SDS) by approximately 40–50%, compared with the untreated control, when examined using lactate dehydrogenase and interleukin-1α levels, indicating the anti-inflammatory effect of phytosphingosine (see Figure 3). In keratinocyte cell cultures, phytosphingosine also reduced the expression of
the pro-inflammatory mediator and pro-inflammatory chemokines such as interleukin-8 (IL-8), CXCL2 and endothelin-1 (data unpublished).

Most recently, phytosphingosine has been shown to inhibit histamine-induced scratching and vascular permeability following its topical application. This data suggests that phytosphingosine might be useful for the treatment of sensitive skin of which body itching is a component, as well as the itchy scalp that occurs in dandruff.

**Phytosphingosine as an Antimicrobial**

While Wertz and Downing were the first to identify free sphingoid bases in the SC, it was not until the studies of Bibel et al. that they were recognized as important for antimicrobial activity in the SC. Sphingoid bases, including phytosphingosine, were found to be profoundly effective against *Staphylococcus aureus*, *Micrococcus luteus*, *Propionibacterium acnes*, *Brevibacterium epidermidis* and *Candida albicans*, while moderately active against *Pseudomonas aeruginosa*. Neneoff and Haustein also demonstrated that phytosphingosine inhibited the growth of *Malassezia furfur* and *Candida albicans*. Further, Pavicic et al. also demonstrated that even at low concentrations, phytosphingosine inhibited the growth of Gram-positive and Gram-negative bacteria, yeasts and molds. The lowest concentration required for growth inhibition within 1 hr was observed for *C. albicans* (0.0012%) and the highest was for *E. coli* (0.040%). For *P. acnes*, the concentration of phytosphingosine required for growth inhibition within 1 hr was 0.020%. Figure 4 shows the antimicrobial properties of phytosphingosine with respect to *P. acnes*, which was measured by the number of colony-forming units (CFUs). It was obvious that the outgrowth of bacteria, which occurred at longer inhibition times, was prevented by higher concentrations of phytosphingosine.

The effect of phytosphingosine was also compared with triclosan in an in vivo study. Emulsions were tested on unwashed hands and the percent reduction in microbial counts was determined 1 hr and 4 hr post-product application. After 1 hr, phytosphingosine, phytosphingosine-HCl and triclosan reduced the amount of bacteria on unwashed hands by 68%, 87% and 79%, respectively, and after 4hr, 42%, 68% and 60%, respectively, compared with the effect of the control (0%). The antimicrobial mechanism of sphingoid bases has not yet been sufficiently elucidated and different explanations exist based on such functions as damaging the bacterial cell wall, inhibiting the bacterial protein kinase, bactericidal effects on the cell membrane, and reducing the adherence of bacteria to epithelial cells. The preventive antiseptic effect of the closely related sphingoid base sphinganine has already been demonstrated in patients with healthy skin; sphinganine can cause a significant, two-log reduction in the growth of *S. aureus* and *C. albicans* in the skin. In addition, whereas *P. acnes* plays a pivotal role particularly in its inflammatory variant, phytosphingosine can inhibit its growth with the minimum inhibitory concentration of 0.020%. This clearly could contribute to clinical efficacy.

**Phytosphingosine for Anti-acne**

Excessive sebum production, increased *P. acnes* colonization of the sebaceous gland, ductal epidermal hyperproliferation and aberrant epidermal differentiation, together with inflammation, all contribute to acne. Since phytosphingosine has been shown to influence all of these complications, it should be a useful anti-acne agent and indeed, Pavicic et al. showed phytosphingosine to be a good anti-acne ingredient both alone as well as in combination with benzoyl peroxide.

![Figure 3. The effects of phytosphingosine (PS) on the release of IL-1α after treatment with sodium lauryl sulphate.](image)

![Figure 4. In vivo antimicrobial efficacy of phytosphingosine, phytosphingosine-HCl and triclosan](image)
(BPO). Volunteers with moderate inflamed acne on the face participated in a half-face study with evaluations taken at baseline and days 30 and 60. Phytosphingosine was compared with BPO and a combination of the two. Since the combination of BPO and phytosphingosine in formulations was previously shown to be unstable due to chemical interactions between the actives, the compounds were separated from one another in a two-chamber dispenser. Phytosphingosine alone was found to diminish the number of papules and pustules by 89% but not comedones, while BPO alone decreased papules and pustules by 32%, and comedones by 22%.

However, the combination of 0.2% phytosphingosine with 4% BPO resulted in a synergistic effect with a 72% reduction in comedones and 88% reduction in pustules and papules. Nevertheless, a much faster response was observed; after 30 days of treatment, the number of papules/pustules and comedones was diminished by 60% and 43%, respectively, in comparison with a 25% and 6% reduction with phytosphingosine alone, and 10% and 15% with BPO only. Although phytosphingosine was unable to prevent the formation of comedones, it could control the number of comedones induced, whereas the placebo formula alone could not. Typical results for efficacy of phytosphingosine independently and together with BPO are shown in Figures 5 and 6.

**Formulating for Phytosphingosine Delivery**

Obviously, the delivery of actives into the skin is essential for efficacy, and in

Figure 5. Clinical photographs taken of placebo vs. phytosphingosine (PS) at day 0 and after 60 days
the described studies, no attempts were made to optimize skin delivery. However, Schiemann et al. studied the delivery of phytosphingosine into porcine skin in Franz cells from emulsions containing emollients with differing polarities; one highly polar, including C14-17 alkyl benzoate/PPG-3 myristyl ether; one of medium polarity, including mineral oil and petrolatum; and one nonpolar, including isohexadecane and cyclomethicone. These experiments revealed that the best penetration was achieved using a formulation with a polar emollient mixture, where approximately 35% of the applied phytosphingosine could be detected by high pressure liquid chromatography in the skin. In contrast, only 18% of the phytosphingosine penetrated into skin with a formulation using medium polarity emollients, and nearly no phytosphingosine was detected in skin using the nonpolar formulation (see Figure 7).

According to Wiechers et al., three factors must be taken into account to estimate the penetration of an active into the skin: first, the polarity of the active; second, the polarity of the SC; and third, the polarity of the formulation. This is partially in line with the results of Schiemann et al., which clearly show that a variation of the polarity of the emollient in the formulation has an extreme impact on the bioavailability of the active.

In the case of phytosphingosine, the best penetration results were observed with the formulation containing C14-17 alkyl benzoate and PPG-3 myristyl ether, which is a polar emollient mixture having a log P o/w value that is somewhat higher but in the range of the SC and the active. The authors propose that this polarity favors penetration into the skin, partitioning the active from the formulation and diffusing into the skin. A higher difference in the polarity between formulation and active/SC decreased the penetration rate by 45% and thus the bioavailability significantly, which was shown by the mineral oil and petrolatum emollient system. Finally, incorporation of the dimethicone prevented penetration almost completely.

To further prove that increased skin delivery aids efficacy, the same polar and nonpolar cosmetic formulations containing phytosphingosine were topically applied on skin models and the biological activity of involucrin, keratin-10, profilaggrin, transglutaminase-1, loricrin and filaggrin was determined by RT-PCR. The expression of all respective genes appeared to increase when a formulation with polar emollients was used (see Figure 8). The right formulation system thus will exploit the different activities of phytosphingosine and further experiments are planned to demonstrate these effects in vivo.

**Conclusion**

The studies described here demonstrate the multiple benefits of phytosphingosine as a skin-identical sphingoid base. It clearly possesses antimicrobial activity, anti-inflammatory activity and aids the differentiation of keratinocytes. The authors propose the material’s anti-inflammation and differentiation activities are related to
its property as a pan-PPAR agonist. As such, phytosphingosine is likely to have many positive skin benefits. Indeed, clinically it has been proven effective as an anti-acne agent when used alone or in combination with traditional anti-acne agents. Further evidence has been presented to improve the delivery of phytosphingosine by use of polar emollients in skin care emulsions; this approach will likely satisfy the consumer need for natural skin care.

Figure 8. RT-qPCR results of reconstructed human skin treated with phytosphingosine in formulations with different emollient polarities; white column = unpolar; black column = polar; given are means ± SEM with * for p ≤ 0.1.

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