Antimicrobial and Anti-inflammatory activity and efficacy of phytophosphine: An *In vitro* and *In vivo* Study Addressing Acne Vulgaris

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**Abstract**

In acne different pathogenetic factors contribute to the inflammation process, defect in keratinisation, increased sebaceous gland activity and increased colonisation of Propionibacterium acnes. The results of *in vitro* and *in vivo* studies confirm the previous reports on strong anti-microbial effectiveness of phytophosphine *in vitro* and *in vivo*. In addition, phytophosphine shows excellent clinical results in the context of skin care in acne, based on the two properties, i.e. anti-inflammatory and anti-microbial activity. These results demonstrate the potential of phytophosphine to enhance or complement existing acne therapies acting as an active cosmetic ingredient.

**Introduction**

The earliest subclinical acne ‘lesion’ is a microcomedone, hyperproliferation of the follicular epithelium being its characteristic feature. Recently significant pro-inflammatory factors, such as interleukin-1, have been identified around clinically normal pilosebaceous follicles from uninvolved skin in acne patients prior to hyperproliferation of the follicular epithelium[1]. This contributes to the concept that acne vulgaris should be classified as an inflammatory skin disease.

**Materials and methods**

I *In vitro* studies

**Methods**

**Anti-microbial activity**

Using a methodology similar to that previously described(2) the inhibitory effect of phytophosphine on growth of different micro-organisms was tested.

**Release of interleukin-1α by UVB irradiated human skin on culture**

The effect of UVB was investigated using human skin explants in culture as a model. Phytophosphine (0.2% and 1.0%) and dexamethasone (10M-6) were applied to human skin explants in culture to test their anti-inflammatory potential. The products were applied one hour before and immediately after irradiation (20 minutes of UVB 2 J/cm²). The interleukin-1α secretion was measured using an ELISA kit at 24 hours.

**Effect on the Artificial Human Epidermis after Irritation with SDS**

The efficacy of phytophosphine on a 3D artificial skin model (SkinEthic™) was investigated after damage with the irritant surfactant sodium laurylsulfate (SDS). After thawing of the artificial human epidermis followed by controlling their viability, a 0.25% SDS solution (dissolved in PBS) has been added to the skin models for 40 minutes to induce chemical stress.

Afterwards the skin slides were washed and a cosmetic O/W formulation (vehicle, formulation containing 0.145% phytophosphine) was applied.

After 24 hours different parameters as cell death represented by lactate dehydrogenase (LDH), viability according to the XTT assay, inflammatory response judging from interleukin-1α (IL-1α) expression.

II *In vivo* studies

**Topical in vivo study on anti-microbial efficacy**

The anti-microbial efficacy of topical phytophosphine within an emulsion-based format was determined in an *in vivo* test. Two products (phytophosphine and phytophosphine-salt) were compared against a control formulation, and a frequently used anti-microbial, triclosan, as a positive control. The formulations were tested on the unwashed hands of 12 subjects based on bacterial counts. The total microbial count was redetermined on the skin at zero time, after 1 hour and after 4 hours.
Active Ingredients

Clinical study on acne skin
The randomised, half-face trial was performed in three separate dermatology centres based in France. There were two different parts to this: in the first the effectiveness of phytosphingosine with benzoyl peroxide was compared to that of benzoyl peroxide alone, in the second phytosphingosine was tested versus a placebo.

Subjects
In the first part of the trial 15 non-pregnant women and 15 men with an average age of 20 years and moderate inflamed acne were enrolled after written informed consent. In the second part of the trial 15 volunteers – 7 women and 8 men - aged between 10 and 50 years participated.

After face cleansing in the morning and evening, the first group received on the right face side benzoyl peroxide (Brevoxyl®), and on the left face side a combination of phytosphingosine and benzoyl peroxide from the Symbio® dispenser. In the second group the right side of the face was treated with the placebo preparation (Brevoxyl® like formulation without benzoyl peroxide) and the left side with phytosphingosine, combined with a Brevoxyl® like formulation without benzoyl peroxide in Symbio® dispenser, also twice a day. The products were applied for the duration of the whole treatment period of 60 days. Each patient was requested to return the first two dispensers used after 30 days.

Measurements
The dermatologists evaluated the results on day 0, day 30, and day 60. The objective criteria were the number of comedones as well as papules and pustules on the left and right side of the face. The intensity of the acne was repeatedly determined using the scale value based on the scale ECLA.

Results
1 In vitro studies
Anti-microbial activity
Phytosphingosine inhibited - even at very low concentrations - the growth of Gram-positive and Gram-negative bacteria, yeasts and moulds. For Propionibacterium acnes the concentration of phytosphingosine required for growth inhibition within one hour was 0.020%. The graph below presents the anti-microbial properties of phytosphingosine with respect to Propionibacterium acnes by measuring the number of CFUs (Figure 1). It became obvious that the outgrowth of bacteria, which occurred at longer inhibition times, is prevented by using higher concentrations of phytosphingosine.
Release of interleukin-1α by UVB irradiated human skin in culture

In the non-treated UV-B exposed skin the IL-1α release was increased by a factor of 4.2 compared to the non-irradiated skin. Compared to the non-treated UV-B exposed skin, dexamethasone, 0.2% and 0.1% phytosphingosine inhibited the release of IL-1α markedly, figures reading 56%, 78% and 72%, respectively (p<0.05). The anti-inflammatory properties of phytosphingosine were almost the same for the 0.2% and 0.1% concentration.

II In vivo studies

Topical in vivo study on anti-microbial efficacy

A statistically significant difference of p=0.078 at 1 hour and p=0.02 at 4 hours was detected between the control and the other groups. Phytosphingosine, phytosphingosine-salt and triclosan has reduced the amount of bacteria on unwashed hands by 68%, 87% and 79% respectively 1 hour post product application compared to the effect of the control (0%).

Phytosphingosine, phytosphingosine-salt and triclosan compared to the effect of the control has reduced the amount of bacteria on unwashed hands by 42%, 68% and 60% respectively 4 hours after product application (Figure 2).

Clinical study on acne

At day 30 benzoyl peroxide reduced the number of comedones by 15%, the combination product by 43%. At day 60 the reduction of 22% respectively 72% was achieved (Figure 3).

The effect on papules and pustules was even more impressive. Benzoyl peroxide alone reduced their number by 10% at day 30, respectively by 32% at day 60. Combination with phytosphingosine had a much stronger effect, 60% and even 88% reduction at day 60 being found (see Figure 3 overleaf).

Group 2: phytosphingosine versus placebo

With placebo use the number of comedones increased by 43% according to the data obtained on day 30 and 60. Phytosphingosine was able almost to control the development of new comedones. Only an increase of 6% was seen.

Papules and pustules were not influenced by the placebo. The preparation containing phytosphingosine almost reduced the number of papules and pustules at day 60 to zero, exactly by 89% (see Figure 4 overleaf).
Active Ingredients

Figure 3 Clinical photographs taken on day 0 and after 60 days in the first treatment group: Benzoyl peroxide versus benzoyl peroxide plus phytosphingosine

Before application

After 60 days

BPO (3.7%)

BPO (3.7%)
+PS (0.2%)

Placebo

Placebo
+PS (0.2%)

Figure 4 Clinical photographs taken on day 0 and after 60 days in the second treatment group: Placebo versus phytosphingosine
Conclusion
Phytosphingosine is a lipid occurring naturally in the stratum corneum, both in its free form and as part of the major fraction of ceramides. The data presented in this study provide novel evidence for the anti-inflammatory and anti-microbial activity of phytosphingosine. These two anti-acne properties work synergistically resulting in a good effect on the clinical status of acne-prone individuals. This in particular applies to the inflammatory type of manifestation. Furthermore the idea of an active ingredient having both antimicrobial and anti-inflammatory activities is exciting, and forms the rationale for the development of the topical phytosphingosine formulation tested in our in-vivo study.

References

Author Biographies
Dr Mike Farwick studied at Heinrich-Heine University Düsseldorf from 1988 to 1994 and obtained a PhD in Molecular Biology in 1997. He worked in Degussa’s Feed Additives business unit from 1997-2003 as Laboratory Head New Technologies, responsible for DNA-Chips, Proteomics and Bioinformatics. Since 2003 he has been responsible for R&D Active Ingredients in Degussa Goldschmidt’s (now renamed Evonik Goldschmidt) Personal Care business line. Dr Farwick’s fields of activity are: in vitro claim support with a focus on molecular processes using DNA-Chip technology and other “omics”; penetration and stability analysis of Actives; encapsulation technologies and in vivo studies for the demonstration of the cosmetic efficacy.

Betty Santonnat has an engineering degree in Biology from the Industrial Biology School near Paris. She began her career in the cosmetic industry 10 years ago when she joined the Dutch biotechnology company Cosmoferm. Originally responsible for technical support for active ingredients, she has weathered acquisitions by Goldschmidt and Evonik industries (formerly Degussa). For the last 2 years, she has been the Global Marketing Manager of the product line Actives.