Salicyloyl Phytosphingosine – The Next Generation Anti-Aging Active Ingredients

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a: Before application of the Salicyloyl Phytosphingosine containing cream;
b: After 4 weeks of application;
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Keywords: Salicyloyl Phytosphingosine, anti-aging, photo damage, dermis, dermal-epidermal junction, collagen

Abstract

The article describes the anti-aging efficacy of Salicyloyl Phytosphingosine. Particularly its effects on dermal level such as on procollagen-I synthesis in fibroblasts in vitro, its effects in vivo on the expression of dermal markers such as fibrillin, procollagen-I and matrix metalloprotease 1 (MMP 1) and finally its effects on periorbital wrinkle reduction over four weeks using the FOITS method is demonstrated. In vitro a significant increase in the production of procollagen-1 by adult human fibroblasts could be observed. In vivo test products were applied under occlusion prior to biopsy and histological assessment in photo-aged volunteers. Increased deposition of fibrillin and procollagen-I together with reductions in the levels of MMP 1 were observed for the Salicyloyl Phytosphingosine treatments. Finally it was evaluated the efficacy of Salicyloyl Phytosphingosine in an O/W cream for its effects in reducing the appearance of periorbital wrinkles in a four week study compared to vehicle. Clear reductions in the Rz and Ra parameters were observed indicating an anti-wrinkle benefit. The 2% solution of Salicyloyl Phytosphingosine in PPG-3 Myristyl Ether is a novel anti-aging active ingredient which can be easily incorporated into the oil phase of both O/W or W/O emulsions.

Introduction

With growing lifespan of the society life style especially of elder people changed drastically during the last decades. Senior citizens live an active life, practice sports and care for a balanced nutrition. Early aging should be delayed or even reversed (1). That includes particularly the skin as the outer appearance and a representative mirror of its wearer. As a consequence, keeping the skin in a «younger status» begins in the 20’s where the process of aging should be postponed. Within the 30’s the first signs of aging like expression lines should be kept whereas during the 40’s deeper wrinkles and other signs of aging should be inverted. Mature women in the 50’s try to counteract the effects of hormonal changes during menopause. So, in the Skin Care market anti-aging products are key drivers due to consumers concerned about skin aging.

Skin Aging

Skin is a highly complex tissue with lots of different functions which underlie drastic changes in both structure and chemistry during aging. These genetically programmed changes start from the early 20’s on, where skin enters a long process of aging (chronological aging, «simply getting older»):

- The epidermal skin renewal is slowed down and disrupted due to a decelerated migration of keratinocytes. The skin becomes dryer, rougher, dull and fragile.
- Due to reduced stratum corneum (SC) natural moisturising factors (NMF) and shortened levels of barrier lipids dry skin occurs more frequently and the skin finally becomes less hydrated and moisturized.
- The dermal-epidermal junction (DEJ) connecting the epidermis with the underlying dermis gets thinner and the nutrition from dermis to epidermis is less supported.
- In the dermis reduced synthesis of collagen as the major structural protein occurs and an increase in the levels of matrix metalloproteases (MMP’s) leading to a degradation of the dermal matrix. Break down and the decreased amount of collagen and other elastic fibers finally cause a loss of elasticity and a sagging of the skin. Reductions in endogenous hormone levels can al-
so contribute to a decomposition of collagens during the menopause caused by reduced concentrations of estrogens.

The consequence of chronological aging is actually made even worse by UV irradiation which leads to photo-aging (premature skin aging). UV irradiation can directly damage cellular membranes leading to reactive oxygen species which initiate the photo-aging process followed by increased levels of inflammatory cytokines such as interleukin-1α. Symptoms of photo-aging are age spots and wrinkles but even changes in non-UV exposed skin occur as a loss of skin firmness and elasticity. Sensitive subjects like individuals with pale skin (phototypes I and II) generally perceive the skin changes earlier than subjects with coloured skin (2). Life style factors (e.g. habits, diets, smoking) and diseases such as diabetes also contribute to the extent of the aging problem.

**Active Ingredients with Anti-Aging Efficacy**

To prevent or slow down the aging process the skin should be provided with active ingredients supporting and stimulating the natural metabolism of the overall skin from stratum corneum down to the dermis. Besides the reduced epidermal skin renewal and metabolic performance, in the dermis a breakdown of collagen and elastin fibers occurs finally leading to a loss of elasticity. As a consequence, wrinkles become visible and the skin sags. Furthermore, the dermal-epidermal junction (DEJ) begins to thin and the nutrition and interaction between dermis and DEJ is less supported. Due to the reduced metabolic efficiency and biochemical changes in collagen and elastin a support of dermal protein synthesis by active ingredients would improve the skin’s condition like firmness and elasticity. So, chosen anti-aging active ingredients should stimulate the dermal and epidermal metabolism, impact the signalling and reverse finally the appearance of «aging» by addressing decreases in key protein and lipid amount and by minimizing levels of certain by-products of UV exposure.

**TEGO® Derm CBS**

Due to the identified efficacy of sphingolipids to influence biochemical processes via signal transduction (3, 4) and the known property of salicylic acid and its derivatives to reduce signs of aging (5) a combination of a sphingolipid with salicylic acid should lead to a sophisticated active ingredient especially suitable for anti-aging products. TEGO® Derm CBS is a 2% solution of Salicyloyl Phytosphingosine in PPG-3 Myristyl Ether. In detail, Salicyloyl Phytosphingosine represents a particular synthetically-derived sphingolipid coupled with salicylic acid (Fig. 1) whereas PPG-3 Myristyl Ether is an emollient with the capability to enhance skin penetration and therefore efficacy. The combination of the two components leads to a novel active ingredient designed for a complete and efficient anti-aging response.

The influence of Salicyloyl Phytosphingosine on the biochemical processes in skin cells could already be demonstrated with the help of DNA Chip analysis (6). More than 350 genes were identified regulated significantly after exposure to Salicyloyl Phytosphingosine. Based on these data which showed stimulation on keratinocyte differentiation after application of Salicyloyl Phytosphingosine (6), several studies regarding its efficacy on epidermal level were performed (7).

The present article focuses on the anti-aging activity of Salicyloyl Phytosphingosine in the dermis. Therefore, at first the efficacy of Salicyloyl Phytosphingosine on procollagen-I synthesis in human fibroblasts was tested in vitro. Primary human adult dermal fibroblasts were cultivated to sub-confluency in 96 well plates and the culture medium was spiked with 10 µM Salicyloyl Phytosphingosine or ethanolic vehicle only. After 48 hours incubation supernatants were removed and assayed for Procollagen-I C-Peptide (PIP).

The effects of Salicyloyl Phytosphingosine on adult dermal fibroblasts is shown in Fig. 2 where the results are expressed as ng PIP / me / supernatant. Biochemical analysis (Enzyme-linked immunosorbent assay ELISA) demonstrated a 54% increase in the levels of procollagen-I found in the supernatant culture medium of the fibroblasts. To confirm these findings the influence of Salicyloyl Phytosphingosine on symptoms of photo-aging as the combination of chronological aging and the effects of cumulative UV exposure has been determined with the help of skin biopsies. It is well-known that photo-aging causes destruction and loss of extracellular matrix (ECM) constituents at the dermal epidermal junction (DEJ) and in the dermis. Many studies have shown that type I collagen levels in skin decreases with age and/or with increased photo-aging (8), partly by the influence of matrix metalloproteases (MMPs) as collagen-degrading enzymes.

For the study, ten healthy but clinically photo-aged volunteers were recruited (54-71 years). Test formulations (Vehicle, 0.2 % Salicyloyl Phytosphingosine and as a positive standard 0.025 % all-trans

![Fig. 1 Salicyloyl Phytosphingosine](Image 263x88 to 547x222)
retinoic acid (9)) were applied separately under occlusive patch (6 mm Finn chambers) to the extensor aspect of the forearm. Therefore, the test formulations consisting of a mixture of propylene glycol, ethanol and water were applied to clean skin on days 1 and 4 of the assay except all-trans retinoic acid which due to potential side effects was applied on day 4 only. On day 8, Finn chambers were removed and 3 mm punch biopsies were obtained from each test site. Biopsy sections were frozen and finally prepared for immunohistochemistry. The degree of immuno staining was assessed with a semi-quantitative score (0 = no staining; 4 = maximal staining). As marker proteins to estimate the grade of photo-aging fibrillin, procollagen-I and MMP 1 were chosen. Fibrillin is a constituent of the elastic fiber network, located and acting at the DEJ. It probably anchors epidermis to dermis. In photo-aged skin the fibrillin amount is drastically reduced (10) contributing to wrinkles and laxity. Procollagen-I as the precursor of collagen I is an indicator for dermal collagen synthesis. The amount of MMP 1 as a collagen-degrading enzyme is increased by the influence of UV irradiation finally resulting in a decrease of dermal collagen. Reduction of its efficacy or quantity will lead to less collagen degradation and thus to an increased dermal collagen content.

Fig. 3 summarizes the outcome of the biopsy study. Typical immunohistochemical images (Magnification 40x) for fibrillin, procollagen-I and MMP 1 are shown. Fig. 3 demonstrates that the application of 0.2% Salicyloyl Phytosphingosine increases by 82% fibrillin 1 synthesis and...
a reinforcement of the fibrillin-filled microfibrils down from the DEJ was observed (See arrows).

Regarding procollagen-I the grey staining of the papillary dermis demonstrates that the procollagen-I content increased by 30% whereas all-trans retinoic acid only showed little effect in this shortterm study.

Additionally, the application of Salicyloyl Phytosphingosine reduced the amount of the MMP 1 in the dermis by 46%. In contrast, the application of all-trans retinoic acid showed no effect on MMP 1 expression in the short-term study.

The results obtained by skin biopsies demonstrate that Salicyloyl Phytosphingosine reduces effectively signs of photo-aging.

To confirm the anti-aging efficacy verified in the dermis and at the DEJ, an in vivo study with the FOITS equipment was conducted.

The »Fast optical in vivo topometry of human skin« (FOITS) is a contactless method to measure the three-dimensional profile of skin areas and therefore to estimate the influence of active ingredients on wrinkles. The FOITS measuring system consists of a projection unit projecting a grid on the skin areas and a fixed camera visualizing the surface curvatures of the detected skin sections. Changes in skin profile can be quantified via the measurement of approx. 50 singular lines perpendicular to the mainstream of wrinkles beginning near to the eye.

For the FOITS study thirty volunteers applied twice daily an O/W cream without (vehicle) and with 0.2% Salicyloyl Phytosphingosine during a period of four weeks to their periorbital areas of the face (half-side test). The facial side for the application of the two products was randomized. Volunteers were required not to use any other cosmetic product 3 days prior and during the application period. The measurement of the skin profile was carried out in a climatic room with defined conditions of 22 °C and 50% relative humidity before and after 4 weeks of application. To acclimate the volunteers staid for 45 minutes in the climatic room prior to the measurement.

The singular profile differences are displayed by the Rz value which is defined as the mean value thereof. A smoothing effect related to the macro structure of the skin is expressed by decreased Rz values. The Ra parameter stands for the fine structure of the skin due to its definition as the arithmetic mean value of all deviations of the roughness profile beyond the axis. A smoothing efficacy and an improvement of the fine structure is represented by decreased Ra values.

Fig. 4 illustrates the excellent efficacy of Salicyloyl Phytosphingosine in reducing the parameters Ra and Rz related to initial value and vehicle. The results obtained by the FOITS method were substantiated.

Fig. 4 Improvement of skin profile related to initial value and vehicle

Fig. 5 Representative photographical analysis of the periorbital skin structure

a: Before application of the Salicyloyl Phytosphingosine containing cream;  b: After 4 weeks of application;  c: Profile width (black: Baseline, red: After 4 weeks)
by a photographic analysis of the skin structure before and after the application period as well (Fig. 5). The representative case study shows that Salicyloyl Phytosphingosine reduces the depths of wrinkles significantly and therefore smooths the skin’s appearance obviously by restructuring the fine structure and improving the macro structure.

As a 2% solution of Salicyloyl Phytosphingosine in PPG-3 Myristyl Ether, TEGO® Derm CBS is a novel active ingredient designed for a complete and efficient anti-aging response. PPG-3 Myristyl Ether is an emollient enhancing the skin penetration of Salicyloyl Phytosphingosine (Data not shown).

TEGO® Derm CBS can easily be incorporated into the oil phase of O/W or W/O emulsions. Recommended use concentrations are between 2 and 10%. As TEGO® Derm CBS is a pumpable liquid, it can also be used in cold processed formulations. A guideline formulation of a hot processed cream is shown in Fig. 6.

## Conclusion

Anti-aging active ingredients for Skin Care are highly demanded due to a society getting older. Research seeks for active ingredients with a broad efficacy spectrum covering the different layers of mature skin from top (stratum corneum) to bottom (dermis). Especially in the dermis where the decomposition of collagen and elastic fibers are accelerated, potent active ingredients are needed which are able to penetrate effectively down into the dermis to support the metabolism in situ.

The present article describes the influence of TEGO® Derm CBS on several biochemical key processes in the dermis. It could be shown that TEGO® Derm CBS increases the procollagen-I synthesis in human dermal fibroblasts. In photo-aged skin it increased the procollagen-I amount and improved the microfibrillar network proximal to the dermal-epidermal junction. Furthermore, the FOITS study demonstrated the skin-firming efficacy of TEGO® Derm CBS.

Additionally, TEGO® Derm CBS is able to support and improve epidermal biochemical processes such as keratinocyte differentiation and skin barrier generation (Data not shown). So, the holistic efficacy of TEGO® Derm CBS successfully reduces the signs of chronological and photo-induced aging by performing an overall anti-aging activity and as a «collagen boosting system» it initiates the next generation of anti-aging active ingredients.

### References


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