An exemplary claim substantiation for the firming / filling activity of a synthetic tripeptide

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Tetradecyl-Dab-Val-Val (HCl: Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetate) is a tripeptide with anti-ageing effects in skin. In its product form it is combined with water, gliceryne, and magnesium chloride (DSM product code 505133134), and marketed under the trade name SYN®-HYCAN. It thus belongs to DSM’s SYN-peptide family. Its sequence is derived from the activation domain of thrombospondin-1 and modeled by rational design to activate transforming growth factor beta (TGF-β). Its main activity in vitro is the stimulation of the skin’s endogenous synthesis of glycosaminoglycans, in particular hyaluronic acid (HA), and proteoglycans, in particular decorin and lumican [1]. There is a decrease in glycosaminoglycans with chronological as well as photo-ageing [2]. Dermal fillers, like collagen or hyaluronic acid fillers, are high on the agenda for many women to counteract cutaneous atrophy [3] leading to facial wrinkles, loss of skin firmness, and skin sagging. However, such fillers require beauty doctor’s visits and needle injections. This tripeptide, though, provides an easy to use cosmetic solution. The data presented in this poster are from new clinical and pre-clinical studies. They provide further evidence for the tripeptide’s outstanding activity for facial skin firming and moisturization [4] by acting as a needle-free hyaluronic acid booster. We propose that Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetate (hereafter called tripeptide) acts particularly in areas below the eyes, on the cheeks, and on the jawline [1].

Introduction

In vitro study: This was a full-face, base formula controlled, randomized single center study. It took place from October 18th to December 15th, 2017, in Schenefeld-Hamburg, Germany. We recruited female Caucasian volunteers age 41 to 60 (mean 52.9 ± 4.8 years) with self-perceived sagging of the skin. We had three cohorts (Base, Active, Benchmark) consisting of 30 volunteers each. Volunteers were instructed to apply their respective formulation twice daily to the entire face for 29 days. Instrument measurements to assess various skin parameters were done at baseline, day 15, and day 29. Composition of the three formulations is listed here:

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We investigated the hyaluronic acid boosting, and firming / filling activity of the synthetic tripeptide Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetate (trade name SYN®-HYCAN) in vitro, ex vivo and in vivo. We provide further evidence, that the tripeptide stimulates endogenous hyaluronic acid synthesis in facial skin tissue both in vitro and ex vivo, also after UV-irradiation. In vivo we show that this leads to a firming and firming effect similar to hyaluronic acid injections at distinct facial sites. In addition, treatment with a formulation containing the tripeptide leads to increased cutaneous hydration, possibly due to the high water binding capacity of hyaluronic acid [4].

Material & Methods

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Skin firmness was measured by Dynaskin (Estecl, France). An air stream is blown perpendicularly to a defined spot on the cheek. Depth, circumference, and negative volume of the as such generated whole in the cheek are measured.

Skin filling was measured by acquisition of 3D facial scans using the AEA-HE device (Estecl, France). Images were processed and using a defined algorithm positive volume was assessed.

Skin hydration was measured by Corneometer (Courage & Kazaka, Germany). Five distinct spots each on right and left cheekbone were measured and mean values calculated.

In vitro study: We grew 3D-microtissue (microtissues) resembling human skin. They consisted of a fibroblast core (dermis) and an outer keratinocyte layer (epidermis). The skin microtissues were treated for ten days with the active (consisting of the peptide product) and a positive control (basicFGF). Culture medium was replaced every other day. After ten days skin microspheres were harvested, frozen fixed and paraffin-embedded. They were cut into enronch tissue sections for staining of hyaluronic acid by alcian blue Kit, Chroma BGS, AppliChem GmbH, Germany.

Ex vivo study: Abdominal skin samples from a Caucasian female donor age 46 were harvested after obtaining informed consent and following Helsinki declaration. The skin simulator adopted for daily UV-irradiation was a BIO-SUN system produced by Vilter Lourmat (Eberhardzell, Germany). The selected UV irradiation intensity was 3 J/cm² (50.1 J/cm² UVB + 2.9 J/cm² UVA) corresponding to 40x biologically effective dose (BED) for daylight UV [5]. Hyaluronic acid was stained by alcian blue (Alcian Blue BGS staining kit, Sigma). AdRit cat 9A157. Semiquantitative evaluation of staining intensity was done by Image J software (NIH, Bethesda, US).

Results in vivo

Figure 1: (A) Face of a young woman, (B, C) She has an augmented cheekbone area (fields 1 and 2), firm cheeks (field 3), and a well defined jawline (field 4). Fields 1 to 4 denote areas of filling mainly to lift or augment the cheek area. Hyaluronic acid filling in these four facial areas is often done in plastic surgery to achieve a well defined young looking contour.

Figure 2: (A) A formulation containing 2.5% Active led to increased volume as measured by AEA-HE and distance per pixel in the area below the eyes of volunteers. A formulation containing 2.5% Active led to a significant increase in volume compared to base formulation. *p<0.05 vs baseline. (B) 3D false color images of the area below the eyes (field 1). Displayed are images of volunteers representing approximately the mean values shown in A. (C) Volume measured by AEA-HE 3D-image scans. A formulation containing 2.5% SYN-HYCAN led to a significantly increased volume compared to baseline after 29 days. *p<0.05 vs baseline. Error bars represent standard error of the mean.

Figure 3: (A) A formulation containing 2.5% Active led to improved firming as measured by Dynaskin on the cheeks of volunteers. It even outperformed a formulation containing a market relevant anti-aging technology (Benchmark). *p<0.05 vs benchmark. *p<0.05 vs baseline. Error bars represent standard error of the mean. (B) 3D false color images of a cheek hole generated by the Dynaskin airstream. Depth, but also circumference clearly improved after 29 days of active formulation. A representative volunteer is shown. Depth and circumference result in negative volume.

Figure 4: A formulation containing 2.5% Active led to improved skin hydration as measured by Corneometer on the cheekbone of volunteers. *p<0.05 vs placebo. **p<0.01 vs baseline. Error bars represent standard error of the mean.

Result in vitro

Figure 5: 2.5% active containing the tripeptide stimulated hyaluronic acid synthesis (blue staining) in skin tissue microspheres. BFGF served as a positive control. Shown are representative tissue sections.

Figure 6: 10 ppm tripeptide stimulated hyaluronic acid content in skin tissue ex vivo (blue staining) after UV-irradiation. Retinoic acid served a positive control and the tripeptide served as a negative control reference. *p<0.05 vs vehicle. Error bars represent standard error of the mean.

Summary

We investigated the hyaluronic acid boosting, and firming / filling activity of the synthetic tripeptide Tetradecyl Aminobutyroylvalylaminobutyric Urea Trifluoroacetate (trade name SYN®-HYCAN) in vitro, ex vivo and in vivo. We provide further evidence, that the tripeptide stimulates endogenous hyaluronic acid synthesis in facial skin tissue both in vitro and ex vivo, also after UV-irradiation. In vivo we show that this leads to a firming and firming effect similar to hyaluronic acid injections at distinct facial sites. In addition, treatment with a formulation containing the tripeptide leads to increased cutaneous hydration, possibly due to the high water binding capacity of hyaluronic acid [4].

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