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신규한 Palmitoyl Tripeptide의 피부 주름개선 효과에 관한 연구

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A Study on the Skin Anti-wrinkle Effect of Novel Palmitoyl Tripeptide

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요 약: 콜라겐은 포유동물의 가장 풍부한 동물성 단백질로, 전체 단백질의 약 30 %를 차지하며, 결합 조직에 존재하며 대부분의 장기의 구조적 지지에 기여한다. Tripeptide (glycine-proline-hydroxyproline; INCI name *Tripeptide-29*)는 collagen type I의 주성분이며, palmitoyl tripeptide (palmitoyl-glycine-proline-hydroxyproline; INCI name *Palmitoyl Tripeptide-29*)는 콜라겐의 합성을 촉진하는 항노화 물질로서 디자인된 합성소재이다. 합성된 펩타이드 유도체는 HPLC를 이용하여 분석하였다. *in vitro* test를 통하여 콜라겐합성과 섬유아세포 증식 효능을 확인하였고, 비침습적 기기를 사용하여 피검자에 대한 8주간의 적용결과 피부주름과 탄력의 상당한 개선을 확인하였다. Palmitoyl tripeptide는 우수한 항노화 효능을 갖는 화장품 소재로 사료된다.

Abstract: Collagen is the most abundant animal protein in mammals, accounting for about 30 % of all proteins. It is present in connective tissue and contributes to the structural framework of most organs. The tripeptide (glycine-proline-hydroxyproline) with the INCI name *Tripeptide-29* is main component of collagen type I. The palmitoyl tripeptide (palmitoyl-glycine-proline-hydroxyproline) with the INCI name *Palmitoyl Tripeptide-29* is a synthetic material that was designed as a topical agent to stimulate collagen production. We synthesized the palmitoyl tripeptide as a potential anti-wrinkle compound. This compound has been characterized using HPLC. This compound proved, through *in vitro* tests, to stimulate collagen production and fibroblast proliferation. These results were very promising, so human studies were subsequently performed. We investigated the skin improvement effect of the palmitoyl tripeptide on human skin by using non-invasive instruments. We measured physiological effects such as skin wrinkles and elasticity after volunteers applied the cosmetic products for 8 weeks. We observed significant improvement in skin wrinkles and elasticity after use of the cosmetic products for 8 weeks. We concluded that the palmitoyl tripeptide had an anti-aging effect on human facial skin.

Keywords: *palmitoyl tripeptide, skin wrinkles, skin elasticity, skin density, skin thickness*

1. 서 론

Collagen is the main protein of connective tissue in animals and the most abundant protein in mammals, making up about 25 % to 35 % of the whole-body protein content. It is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging. A distinctive feature of collagen is the regular arrangement of amino acids in each of the three chains of these collagen subunits. The most frequent amino acid sequence in these trihelical fragments is Gly-Pro-X. The most frequent amino acid in the X po-

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sition is Hyp. The peptide Gly-Pro-Hyp makes up about 36 % of the molecular weight of the collagen degradation products mixture, obtained after 6 hours of digestion of trihelical fragments of collagen type I with bacterial collagenase[1]. It was found that the tripeptide component had an acceleration effect on collagen synthesis of dermal fibroblast cells[2].

Although topical use of a peptide has the potential to be effective, delivery across skin can be difficult due to the ionic nature of such materials. An approach to improving delivery is the use of fatty acid derivatives to increase the lipophilic property of the peptide. For example, the palmitoyl derivative of the polypeptide interferon α has been shown to have five to six fold greater penetration across human than the underivatized peptide[3]. The palmitoyl derivative of pentapeptide resulted from research aimed at enhancing peptide delivery while maintaining bioactivity, mildness, and skin benefit potency[4].

In this research, the palmitoyl tripeptide (palmitoyl-glycine-proline-hydroxyproline) with the INCI name *Palmitoyl Tripeptide-29* was designed as a topical agent to stimulate collagen production (Figure 1).

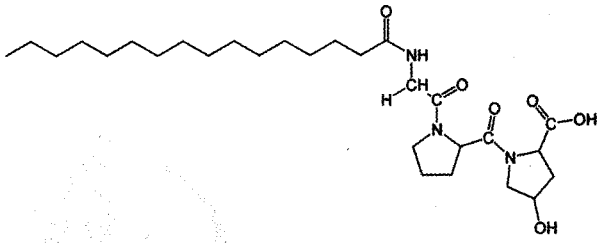

Figure 1. Structure of palmitoyl tripeptide.

2. Materials and Methods

In this study, the palmitoyl tripeptide was synthesized by using NSC-amino acid method. The 2-(4-nitrophenylsulfonyl) ethoxycarbonyl (NSC) group is an alternative to Fmoc for protection in solid peptide synthesis. This compound had been characterized using HPLC and Maldi-Tof mass spectrometry.

2.1. *In vitro* Studies

2.1.1. Determination of Fibroblast Proliferation

In recent years, advancements in techniques have led to cultures that mimic not only the epidermal layer but also the dermal layer, allowing these models to be used not only for safety assessments but also to test product efficacy. Human skin equivalents offer multiple advantages over traditional monolayer cultures. In this study, fibroblasts in suspension are mixed with a bovine type I collagen solution and poured into culture transwells. The cultures are incubated for 1 ~ 2 weeks at 36 ℃, in an atmosphere of 10 % CO_2. During that time the fibroblasts contract the collagen to form a cellular dermal matrix. Human keratinocytes are then seeded onto the surface of the lattice and the construct is submerged for 1 ~ 2 weeks in a medium that allows the keratinocytes to cover the dermal matrix. The fibroblast proliferation was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. MTT solution was added to each well. After incubation, the upper medium was removed carefully and the intracellular formazan was dissolved by adding dimethyl sulfoxide (DMSO) to each well. The absorbance at 560 nm was recorded.

2.1.2. Determination of Collagen Biosynthesis

Collagens (types I, II, III, IV and V) are synthesized as precursor molecules called procollagens. These contain additional peptide sequences, usually called "propeptides", at both the amino-terminal and the carboxy-terminal ends. The function of these propeptides is to facilitate the winding of procollagen molecules into a triple-helical conformation within the endoplasmic reticulum. The propeptides are cleaved off from the collagen triple helix molecule during its secretion, after which, the triple helix collagens polymerize into extracellular collagen fibrils. Thus, the amount of the free propeptides reflects stoichiometrically the amount of collagen molecules synthesized (a relationship analogous to that between the carboxy-terminal peptide of proinsulin and the endogenously produced insulin). We determined collagen biosynthesis by using the Procollagen Type I C-Peptide (PIP) EIA Kit (Takara Bio Inc. USA). The PIP EIA Kit is a solidphase EIA based on

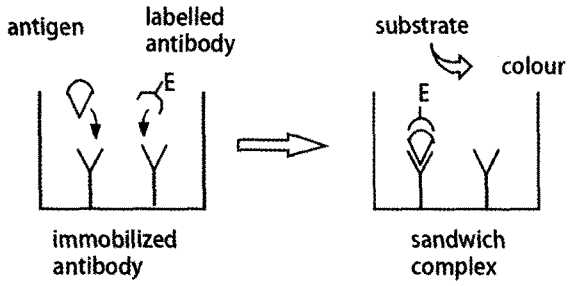

Figure 2. Antigen-Antibody Interaction of PIP Kit.

a sandwich method that utilizes two mouse monoclonal anti-PIP antibodies to detect PIP by one-step procedure. One of the monoclonal antibodies has been pre-coated onto a microtiter-plate and blocked against non-specific binding. Samples, standard, and peroxidase (POD)-labelled anti-PIP antibody are simultaneously added to the wells of plates, and then incubated. During the incubation, PIP is bound to anti-PIP (solid phase) on one side and tagged by POD-anti-PIP on the other. The reaction between POD and substrate (H_2O_2 and tetramethylbenzidine) results in color development with intensities proportional to the amount of PIP present in samples and standards (Figure 2). The amount of PIP can be quantitated by measuring the absorbance using an EIA plate reader. Accurate sample concentrations of PIP can be determined by comparing their specific absorbances with those obtained for the standards plotted on a standard curve.

2.2. *In vivo* Studies

We investigated the skin improvement effect of the palmitoyl tripeptide on human skin by using non-invasive instruments. We measured physiological effects such as skin wrinkles and elasticity after volunteers applied the cosmetic products for 8 weeks. The study was conducted on 15 healthy female volunteers (mean age 44.1 years). The cosmetic products containing 0.01 % of the palmitoyl tripeptide were applied twice-daily on facial skin during 8 weeks. All measurements were performed in a controlled environment room with constant temperature between 20 and 24 ℃ and humidity between 35 and 45%. The anti-wrinkle effect was analyzed by observing the replicas with a Visiometer SV 600, (Courage & Khazaka, Germany). Silicone polymer

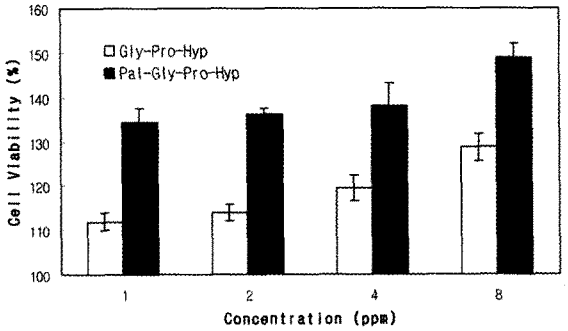

Figure 3. Fibroblast proliferation due to palmitoyl tripeptide and underivatized peptide.

replicas were made of the crow's feet before and after 28 and 56 days. R2 (Maximum Roughness) parameter was studied. Skin elasticity was measured from the crow's feet area with a Cutometer MPA 580 (Courage & Khazaka, Germany). R2 (gross elasticity) parameter was studied. The closer value is to 1, the more elastic the skin is.

Skin thickness (epidermis-dermis) and density were evaluated using 20 MHz ultrasound echography in 8 women (mean age 42.9 years). Measurements were performed by Dermascan C (Cortex Technology, Denmark) on the crow's feet before and after 56 days.

The paired t-test was performed using the Micosoft Excel statistical package. Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Fibroblast Proliferation

The palmitoyl tripeptide induced a linear dose-responsive increase in fibroblast proliferation across a potent range of 1 ~ 8 parts per million (ppm) when tested in cultures of human fibroblast. In comparison to underivatized peptide, a dose of 1 ~ 8 ppm palmitoyl tripeptide was as effective as about 2-fold higher dose of underivatized peptide in fibrobalst proliferation (Figure 3).

3.2. Collagen Production

The palmitoyl tripeptide induced a linear dose-responsive increase in collagen production across a potent

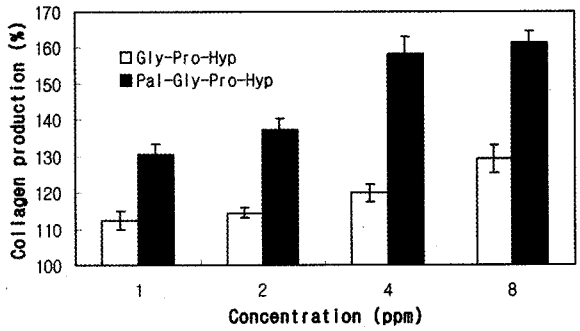

Figure 4. Collagen production due to the palmitoyl tripeptide and underivatized peptide.

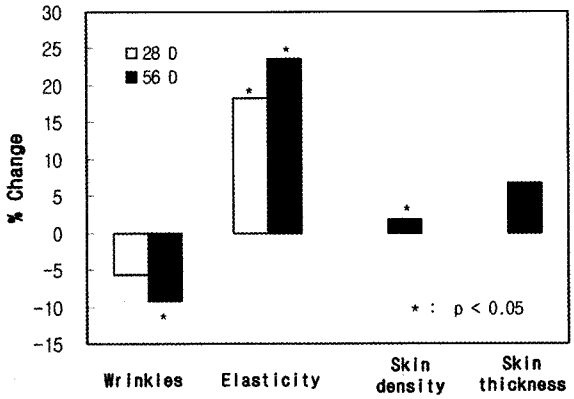

Figure 5. Skin improvement of palmitoyl tripeptide.

range of 1 ~ 8 part per million (ppm) when tested in cultures of human fibroblast. In comparison to underivatized peptide, a dose of 1 ~ 8 ppm palmitoyl tripeptide was as effective as about 2-fold higher dose of underivatized peptide in collagen production (Figure 4).

3.3. *In vivo* Studies

The palmitoyl tripeptide produced significant improvements in skin wrinkles, and elasticity (Figure 5). The palmitoyl tripeptide also was extremely well tolerated, with a low incidence of skin irritation responses such as redness, dryness, burning, stinging, or itchiness.

Moreover, we observed a reduction of subepidermal low echogenic band (SLEB) after using the cosmetic products for 8 weeks (Figure 6). The SLEB is an index of skin (photo)aging level[5]. This layer appears with increasing age. Therefore, a rejuvenation effect on the skin will be expressed by an increase of echogenicity of the SLEB.

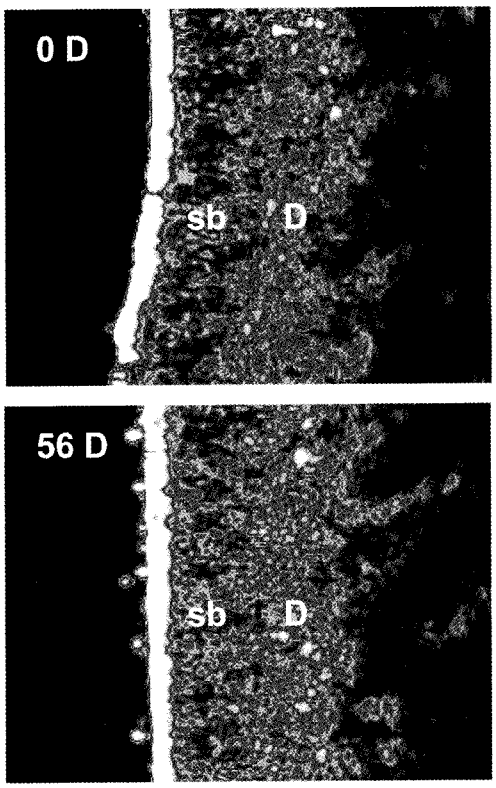

Figure 6. Images of the echographic aspect of the skin (sb = subepidermal low echogenic band, D = dermis.).

4. Conclusion

In this research, we synthesized the palmitoyl tripeptide as a potential anti-wrinkle compound. This compound proved, through *in vitro* tests, to stimulate collagen production and fibroblast proliferation. In cell cultures, the palmitoyl tripeptide showed positive effects on fibroblast proliferation and collagen production. We investigated the skin improvement effect of the palmitoyl tripeptide on human skin by using non-invasive instruments. We measured physiological effects such as skin wrinkles and elasticity after volunteers applied the cosmetic products for 8 weeks. We observed skin wrinkle and elasticity improvement after using the cosmetic products for 8 weeks. In addition, the wrinkles and elasticity of human skin improved significantly with no irritation. We concluded that palmitoyl tripeptide had an anti-aging effect on the human facial skin.

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