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피부 노화 치료로서 저분자콜라겐펩타이드의 피부 항당화와 콜라겐 합성 효과

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The Effect of Low Molecule Collagen Peptide on Skin Anti-glycation and Collagen Synthesis as a Skin Aging Therapy

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요 약: 콜라겐 가수 분해물(collagen hydrolysate , CH)은 피부 진피 섬유아세포를 자극하여 콜라겐과 엘라스틴 같은 세포 외 기질 합성을 촉진함으로써 피부 노화 방지에 도움을 주는 것으로 알려져 있다. 최근 피부 노화를 일으키는 다양한 요인 중 최종당화산물(advanced glycation end products, AGEs)이 주목을 받고 있음에도 불구하고, 아직까지 CH가 AGE축적에 미치는 영향은 연구된 바 없다. CH는 피부 구조단백질의 생성을 촉진하여 AGE 축적에 영향을 줄 수 있으므로, 이를 확인하기 위해 트리펩타이드25%, Gly-Pro-Hyp 4%를 함유한 CH인 저분자콜라겐펩타이드(low molecule weight collagen peptide, LMCP)를 이용하여 임상시험을 수행하였다. 피부 조직 내 AGE 축적량을 평가하기 위해 AGE reader를 사용하여 피부자가형광(skin autofluorescence, SAF) 값을 측정하였다. 0.5%와 1.0% LMCP 용액을 8 주 동안 피험자의 전박에 도포한 결과, 시험부위의 SAF값이 대조부위에 비해 유의하게 감소하였다. 추가적으로, LMCP에 의한 피부섬유아세포의 콜라겐 합성 촉진을 평가하기 위해 CCD-986sk를 이용하여 *in vitro* test를 수행하였다. 그 결과, 800 μg/mL의 LMCP는 CCD-986sk의 human pro-collagen Iα1(COL1A1) 합성을 증가시켰다. 본 연구를 통해 LMCP도포가 콜라겐 합성을 촉진하여 항당화 효과에 도움을 줄 수 있다는 것을 확인하였으며, 이는 LMCP가 노화 방지 화장품 원료로써 잠재력이 있음을 시사한다.

Abstract: Collagen hydrolysate (CH) is known to prevent skin aging by stimulating skin dermal fibroblasts to promote synthesis of extracellular matrix such as collagen and elastin. Recently, among the various factors that cause skin aging, advanced glycation end products (AGEs) have received particular attention. However, the effect of CH on AGE accumulation has not been studied. Since CH could affect AGE accumulation by promoting production of skin structural proteins, clinical trial was performed using low molecule collagen peptide (LMCP), which were CH containing 25% tripeptide and 4% Gly-Pro-Hyp. Skin autofluorescence (SAF) values were measured using an AGE reader to evaluate accumulation of AGE in skin. As a result of applying 0.5% and 1.0% LMCP solutions to the subject's forearm for 8 weeks, the SAF value at the test site significantly decreased compared to the control site. Additionally, *in vitro* test was performed using CCD-986sk

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to evaluate the promotion of collagen synthesis in skin fibroblasts by LMCP. As a result, 800 μg/mL of LMCP significantly increased synthesis of human pro-collagen Iα1 (COL1A1) in CCD-986sk. Through this study, we have confirmed that tropical LMCP applications can promote collagen synthesis to help anti-glycation effects, suggesting that LMCP has potential as an anti-aging cosmetic material.

Keywords: *collagen hydrolysates (CH), low molecule collagen peptide (LMCP), anti-aging, advanced glycation end-products (AGEs), skin autofluorescence (SAF)*

1. Introduction

Collagen hydrolysate (CH) is one of the most popular ingredients in health supplements and a cosmetic product and already well-known for its benefit on skin health. A clinical study has been proved that a long-term oral intake of CH improves skin hydration, elasticity, and wrinkles[1]. The previous study showed that Gly-Pro-Hyp and Pro-Hyp might be associated with wound healing and promote growth in dermal fibroblasts[2,3]. Moreover, CH has been known to stimulate dermal fibroblasts to synthesize new collagen, elastin, and hyaluronic acid[4,5].

In the last decade, advanced glycation end products (AGEs) also have received particular attention in a skin aging-related study. Glycation is the non-enzymatic reaction between proteins and sugars leading to the formation and subsequent accumulation of AGEs[6]. The Maillard reaction, starting from the glycation of protein and progressing to the formation of AGEs, is implicated in the development of complications of diabetes mellitus and the pathogenesis of cardiovascular, renal, and neurodegenerative diseases, as well as skin aging[7]. Recently, in addition to these systemic diseases, AGEs are also known to affect the skin's aging. And identification of AGEs in both the epidermis and dermis in the skin has led to further investigation of the role of glycation in skin aging[8].

Dermal AGEs accumulation is associated with extracellular matrix proteins such as collagen, vimentin, and elastin[8]. AGEs alter the skin's mechanical properties and are therefore associated with skin aging clinical symptoms. In addition, AGEs also affect skin color as well as changes in the skin structure[9].

The glycation process has been known to be very important in aging, and low protein turnover has also been reported to be one of the AGEs accumulation factors[10]. Oral intake of CH has been clinically proven to stimulate the production of new collagen. However, there is not enough study available regarding the relationship between topical CH and anti-glycation.

CH increases collagen production stimulation, we assume it will also increase collagen turnover. Therefore, in the present study, we intended to prove that topical CH reduces superficial AGEs accumulation in skin after 8 weeks of continuous usage in a human model.

2. Experimental

2.1. Ethical Considerations

This study is conducted according to the Good Clinical Practice, relevant regulations of the Ministry of Food and Drug Safety (MFDS), and Human Skin Clinical Trial Center's standard operating procedure (SOP). The institutional review board approved this study protocol of Human Co. Ltd (HM-IRB-0003).

2.2. Low Molecule Collagen Peptide

The low molecule collagen peptide (LMCP), known as the trade name EverCTP (NEWTREE Co., Ltd. Korea) was used as topical CH in this study. LMCP used in the experiment contains 25% tripeptide and 4% Gly-Pro-Hyp. LMCP solutions (0.5% and 1.0%) dissolved in distilled water were used as the clinical trial test products.

2.3. Study Participants and Design

We conducted an open-label study between November 2020 to January 2021. The baseline characteristics of the subject are summarized in Table 1. Subjects were between the age of 31 to 68 years old who met all the inclusion and exclusion

criteria as listed Table 2. All participants are fully aware of the objective, protocol, and possible risk of the study. Participants signed and gave written informed consent for this study.

Subjects are asked to apply both test products on the anterior and posterior side of one forearm while the other forearm was determined to be the control sites. Test product was applied twice (morning and evening) every day for 8 weeks. The amount was adjusted individually according to each participant's needs.

2.4. Skin Autofluorescence Assessment

The invention of the point-of-care device lets us measure AGEs quickly and conveniently through the skin autofluorescence (SAF) technique. In this study, SAF was assessed using the AGEs Reader device (Diagnoptics BV, The Netherlands). The autofluorescence illuminates a skin surface of 1 cm^2 with an excitation light source of 300 ~ 420 nm. The emission light and the reflected excitation light from the skin are measured using a spectrometer (Model PC-1000 fiber optic spectrometer, Ocean Optics, USA). Autofluorescence is calculated as the average light intensity per nanometer in the range between 420 and 600 nm, divided by the average light intensity per nanometer in the range between 300 and 420 nm, multiplied by 100 and expressed in arbitrary units in the range from 0 to 25[11].

Table 1. Characteristics of Subjects

Characteristics	Value (N = 21)
Age (yr)	46.62 ± 8.13
Gender, female	21
Sun exposure	
< 60 min	3
> 60 min	18
Smoking habit	
Smoker	1
Non-smoker	20

2.5. *In Vitro* Study

We analyzed the change amount of collagen 1 synthesis with enzyme-linked immunosorbent assay (ELISA, R&D Systems, DY6220-05, USA). CCD-986sk (skin fibroblast, Korean Cell Line Bank (KCLB), Korea) cells were cultured in an IMDM cell culture medium at 37 ℃ and 5% CO_2 incubator. LMCP with concentration of 800 μg/mL were treated in CCD-986sk cells (1 x 10^4 cells/well) for 24 h. Human pro-collagen Iα1 (COL1A1) content was measured using an ELISA kit and the absorbance was a microplate reader (Synergy H1 hybrid multi-mode microplate reader, BioTek, USA). Absorbance was measured at 450 nm.

Table 2. Inclusion and Exclusion Criteria for Recruitment of Subjects

Inclusion criteria
· Healthy female subjects age 19 ~ 60 years old
· Not restricted to women in recruiting subjects, but not supported by men
· Subjects who have signed consent form voluntarily after being informed sufficiently on the objectives of the study and all related contents
· Subjects who are healthy without acute and chronic diseases, including skin disorders
· Subjects who can be observed and traced throughout the entire study period
Exclusion criteria
· Subjects who are and/or have a plan of pregnant or breast-feeding
· Subjects who have the psychiatric disease and infectious skin disease
· Subjects who have used an ointment containing steroids for more than 1 month
· Subjects who participated in a similar test within the past 6 months
· Subjects who have sensitive and hypersensitive skin
· Subjects who have skin disorders on the test site such as moles, pimples, red spots, scalds (burns), hemotelangiosis, and scars
· Subjects who have used cosmetics or drugs on the test site with similar efficacy within the past 3 months
· Subjects who received treatment from dermatologist or aestheticians on the test site within the past 6 months
· Those who are employed in this clinical trial center
· Those who are considered as a nonqualified person by the judge or the investigator

2.6. Statistical Analysis

Statistical analysis was performed using KoreaPlus Statistics (Embedded on SPSS Statistics 26) Version 26.0.0.0. To evaluate the intra-group's data, Shapiro-wilk test is performed to check if the data satisfy the normality requirement. If the normality is satisfied, *t*-test (parametric method) will be used to verify the data. If the normality is not satisfied, Wilcoxon signed-rank test (non-parametric method) will be used to verify the data. To evaluate the data between control and test group, Shapiro-wilk test is performed to check if the data satisfy the normality requirement. And *t*-test (parametric method) will be used to verify the data.

3. Result and Discussion

CH has been proven to stimulate new collagen production and influence the collagen turnover and balance of different tissues, such as skin or cartilage by oral administration or topical application[4,5,12]. CH not only stimulates skin fibroblasts to promote collagen synthesis, but also down- regulate matrix metalloproteinases (MMPs), the components that breakdown collagen and elastic fibers in extracellular matrix (ECM)[1]. Apart from collagen synthesis, CH have also been shown to stimulate hyaluronic acid (HA) synthesis by activation of *HAS2* transcription in human dermal fibroblasts *in vitro*[1,13].

AGEs accumulation is part of normal human aging, which forms when proteins or lipids interact with aldose sugars for an extended time, subsequently undergoing molecular transformations that glycate the protein or lipid. Indication of AGEs is strongly linked to the body's aging process, starting from life-threatened diseases such as diabetes and cardiovascular disease to skin aging[14]. Preventive remedy of AGEs is important because of its irreversibility once AGEs are formed. Many factors are taking roles in glycation, it could be a genetic and extrinsic factor such as lifestyle, diet, smoking, UV light etc.. In the diabetic mice study, a low AGEs diet has been shown to be effective in controlling systemic AGEs toxicity as well as AGEs deposition in the skin and promoting wound healing effect[15].

Accumulation of AGEs has been found in various tissues during the aging process, including skin, articular collagen, skeletal and vascular smooth muscles, or renal membranes[6]. The skin offers a great opportunity for non-invasive assessment of glycation for early detection of diabetes and vascular-associated disease; investigation with glycation-associated SAF was shown to correlate with chronological aging in a large number of healthy subjects[6,16]. Detection of AGEs in the skin led to more inspection of its association with skin aging.

The AGEs accumulation can induce premature senescence in human dermal fibroblasts and in normal human keratinocytes *in vitro,* decrease the collagen and ECM protein synthesis, and induce the expression of MMPs[6]. The impaired synthesis of collagen and other ECM proteins will eventually contribute to the development of wrinkles. Not only wrinkles, AGEs-related skin aging also caused brown-yellowish skin and loss of skin elasticity[9,16].

Although AGE's role in tackling skin aging still has not been studied much, the anti-AGEs strategy has received high interest from the pharmaceutical company. Since oxidation is a crucial step in forming AGEs, consuming high antioxidant foods or metal-chelating properties is one way to help inhibit AGEs production. Topical treatment containing antioxidants and nanoparticles may be effective in the wound healing process by blocking the receptor for AGEs in diabetic patients[17].

Our study assessed the AGEs level on human skin non-invasively through the SAF technique to assess the product efficacy as a skin anti-aging treatment. To our knowledge, this is the first study that reports the effect of CH on AGEs accumulation.

SAF tends to increase over time, however, our clinical trial result (Table 3) showed a reduce in SAF value on test site. Both 0.5% and 1.0% LMCP solution significantly decreased the SAF of test sites after 8 weeks of treatment. After using

Table 3. Collagen *In Vitro* Study

Assessment \ Concentration	Control	800 μg/mL
COL1A1 (ng/mL)	7.04 ± 0.44	9.19 ± 0.93
p value[1)]	-	0.01
Change percentage	34.62%	

[1)] p value: Significant probability, Paired *t*-test. ($p < 0.05$, comparsion to control group (N = 4).

LMCP 0.5% concentration for 8 weeks, SAF was significantly decreased from 1.94 to 1.83 as shown in Figure 1 and Table 4, respectively. On the other hand, at the control site, SAF had a 7.05% increase from 1.89 to 2.02. As for the other solution, after using LMCP 1.0% concentration for 8 weeks, SAF was significantly decreased from 1.84 to 1.77 as shown in Figure 2 and Table 5, respectively. While at the control site, SAF had increased 12.05% from 1.74 to 1.95. In addition, the SAF of both test sites treated 0.5% or 1.0% LMCP for 8 weeks was significantly lower than the control site.

Therefore, there were quite significant SAF value differences between test and control sites after 8 weeks. Although local treatment might not change the systemic value of AGE, but by reducing SAF value on the skin it will also reduce appearance of AGE-related skin aging. While on the *in vitro* study, LMCP was also shown to increase the stimulation of collagen synthesis and collagen turnover. Our *in vitro* study showed that 800 μg/mL of LMCP significantly increased collagen synthesis of CCD-986sk by 34.62% as shown in Table 3. These results are consistent with the results reported in previous literature that CH promotes collagen synthesis in skin fibroblasts.

This phenomenon could be explained by the relationship between AGEs accumulation and collagen turnover[10]. AGEs level is linearly related to the protein residence time. Therefore, LMCP will increase the new collagen production and collagen turnover, implying a decrease residence time of collagen in the dermis, resulting in a decrease of SAF-linked AGEs accumulation in the dermis. In summary, topical LMCP has a promising result to decrease AGE accumulation by stimulating the collagen turnover in the skin dermis.

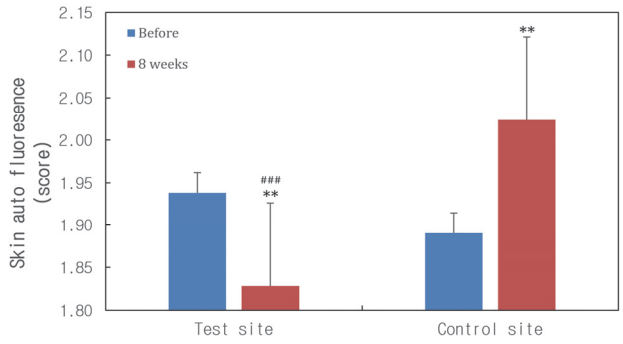

Figure 1. SAF after 8 weeks of using LMCP 0.5%. SAF on the test site showed a decrease while the control site showed an increase. $^{**}p < 0.01$, compared to before treatment. $^{###}p < 0.001$, compared to control site after 8 weeks treatment.

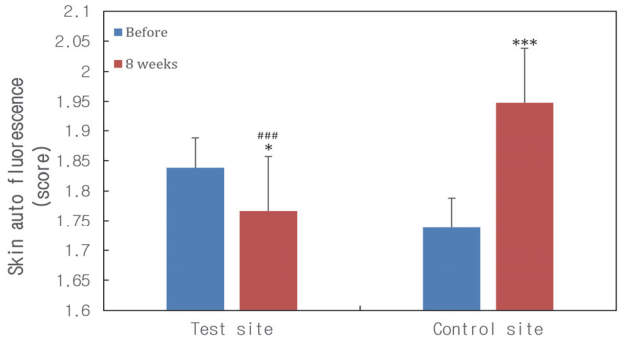

Figure 2. SAF after 8 weeks of using LMCP 1.0%. SAF on the test site showed a decrease while the control site showed an increase. $^{*}p < 0.05$ and $^{***}p < 0.001$, compared to before treatment. $^{###}p < 0.001$, compared to control site after 8weeks treatment.

Table 4. SAF Value after Using 0.5% LMCP

N = 21 (No. 01 ~ 21), (Mean ±SD)

Period \ Area	Test site	Control site
Before (D0)	1.94	1.89
8 weeks (D56)	1.83	2.02
p value (intra-group)	0.007	0.001
Change percentage (D0-D56)	-5.65%	7.05%
p value (inter-group)	0.000	

Table 5. SAF Value after Using 1.0% LMCP

N = 21 (No. 01 ~ 21), (Mean ±SD)

Period \ Area	Test site	Control site
Before (D0)	1.84	1.74
8 weeks (D56)	1.77	1.95
p value (intra-group)	0.015	0.000
Change percentage (D0-D56)	-3.89%	12.05%
p value (inter-group)	0.000	

4. Conclusion

Based on this study, our finidings supported that LMCP has anti-glycation effect by stimulating collagen synthesis, implying LMCP has potential as an anti-aging cosmetic ingredient. Further study is necessary to evaluate the more detailed anti-glycation mechanism of LMCP.

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