Research Article

Clinical Efficacy of Wrinkle Improvement Right After using Antiaging Cosmetics containing Palmitoyl-GHK-R4 Peptide

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Abstract: GHK (gly-his-lys), which was first isolated from human serum and known as a hepatocellular growth factor, is a typical wrinkle improving functional peptide. Pal-GHK is a form of GHK containing palmitate, which increases skin permeability and stability of GHK. However, the problem is that GHK has difficulty in penetrating stratum corneum due to its hydrophilic properties, making it difficult to actually exhibit efficacy in the skin. In this study, Arg 4, one of the skin penetrating peptides, was conjugated with Pal-GHK to get palmitoyl heptapeptide, Pal-GHK-R4, and clinical trials were conducted on the wrinkle improvement effect of cosmetics containing Pal-GHK-R4. As a result of total 21 subjects used the test products on the face area; 1) measurement value of crow's feet statistically significantly decreased right after using the test products compared to before using the products ($p<0.05$); 2) measurement value of skin moisture content statistically significantly increased right after using the test products compared to before using the products ($p<0.05$); 3) measurement value of transepidermal water loss (TEWL) statistically significantly decreased right after using the test products compared to before using the products ($p<0.05$); 4) there were no skin adverse events reported after using the products. Therefore, the test products containing Pal-GHK-R4, which combines wrinkle improving peptide with skin penetrating peptide are considered to have immediate effects on improving crow's feet, skin moisturization, and TEWL.

Keywords: Clinical trials, palmitoyl-GHK-R4, wrinkle improvement, skin moisturization, transepidermal water loss, antiaging cosmetics

1. Introduction

Peptides, which are short chains of amino acids, have outstanding physiological activity, have no side effects because they are substances found in the human body, and can be synthesized because they are smaller than proteins. Thus, peptides have recently attracted attention as functional materials for improving wrinkles in cosmetics (**Moh S.H. et al., 2011**; **Yoo & Yeon, 2017**). GHK (glycyl-histidyl-lysine), first separated from human serum and known as a hepatocellular growth factor, is a typical wrinkle improvement function peptide (**Maquart F.X. et al.,1988**). The level of GHK in the human blood is about 200mg/mL at the age of 20, but it decreases as aging progresses and is about 80mg/mL at the age of 60. This increases the synthesis of extracellular matrix (ECM) such as collagen, elastin, and glycosaminoglycans (GAGs) to regenerate skin and heal wounds (**Maquart F.X. et al., 1993**). GHK generally exists in the form of GHK-Cu due to its high affinity with copper ions, and there is also Pal-GHK, which combines GHK with palmitate, a fatty acid, to increase permeability and stability (**Pickart L., 2008**; **Godet & Marie, 1995**; **Pickart L. et al., 1980**). GHK, GHK-Cu, and Pal-GHK are popular as functional cosmetics materials for wrinkle improvement. However, due to the hydrophilic nature of GHK, it is difficult to penetrate stratum corneum and it is difficult to actually work on the skin (**Li H. et al., 2015**).

Using cell penetrating peptides (CPPs), hydrophilic macromolecules can pass through the cell membranes without destroying cell membranes and showing cell toxicity (**Lee H. et al., 2019**; **Yi D. et al., 2007**). Cell penetrating peptides are generally composed of 10 to 30 short amino acids and mostly contain large amounts of basic amino acids, lysine (K) and arginine (R). TAT peptide derived from HIV-1 is a typical cell penetrating peptide. TAT peptide consists of 86 amino acids, of which the sequence of RKKKRRQRRR is known as the minimum area of penetration of the cell penetrating peptides, among which lysine (K) and arginine (R) play a key role in cell permeability (**Vive E. et al., 1997**; **Wang K. et al., 2019**; **Liang W. et al., 2004**). Both the lipids between the cell membranes and the intercellular lipids are similar to the lipid bilayer structure, so cell penetrating peptides can be applied to the skin penetration of functional materials (**Mazurowska & Mojski, 2008**; **Kim M.H., 2009**; **Landmann L., 1988**).

In this study, clinical trials were conducted on crow's feet, skin moisturization, and transepidermal water loss (TEWL) improvement effects right after using cosmetics containing palmitoyl heptapeptide, Pal-GHK-R4, which combines Pal-GHK with Arg 4 to maximize the efficacy by increasing the permeability of GHK (**Kottner J. et al., 2013**).

2. Materials and Methods

2.1. Study Protocols

The test products used in the clinical trials are four types of Biotoc Regen (Ampoule, Serum, Cream, and Feel) containing 1,000ppm of Pal-GHK-R4. In order to evaluate the improvement effect of the test products on the crow's feet, skin moisturization, and TEWL, a total of 21 subjects who met the selection criteria and did not meet the exclusion criteria was selected. The subjects washed their facial skin with the same cleanser, stabilized it for 30 minutes, and then used the test products once. Photographs and skin measurements were taken before and right after using the products. All the processes were conducted in an indoor environment where the temperature and humidity are constant.

2.2. Crow's Feet Measurement

Crow's feet measurements were taken using Antera 3D before and after using the test products. A specific area of the photo taken was specified and analyzed and used as crow's feet evaluation data. The analyzed wrinkle variable is overall size, and the analysis value and the degree of wrinkle improvement are reverse proportional, which means that the lower the analysis value, the better the wrinkles.

2.3. Skin Moisture Content Measurement

Skin moisturization measurements were taken using Corneometer before and after using the test products. The average value measured three times was used as the skin moisture content evaluation data, and the unit is A.U. (Arbitrary Unit). Since the measurement value and skin moisture content are proportional, the higher the measurement value, the better the skin moisturization.

2.4. TEWL Measurement

TEWL measurements were taken using Vapometer before and after using the test products. The value measured once was used as evaluation data, and the unit is g/m^2h. Since the measurement value and the degree of improvement in TEWL are inversely proportional, the lower the measurement value, the better the degree of improvement in TEWL.

2.5. Statistical Analysis

In order to determine the significance of changes in skin measurement before and right after using the test products, it was verified using SPSS 23.0, a statistical analysis program. Differences were considered statistically significant at $p<0.05$.

3. Results and Discussions

3.1. Measurement Results of Crow's Feet

Crow's feet was measured before and right after using the test products. The overall size of crow's feet was 16.995 ± 4.625 before using the products, but right after using the products, it was decreased to 15.543 ± 4.443 (Figure 1). Analyzing the rate of change by setting the before value at 100%, it decreased by 8.562% right after the use of the products (Table 1). The analysis value of crow's feet revealed a statistically significant decrease right after using the test products compared to before using the products ($p<0.05$). So it was confirmed that there were immediate wrinkle improvement effects when using the test products.

Table 1. Rate of change of crow's feet.

Time	Average ± STD (overall size)	Rate of change [a] (%)	Probability [b] (*p* value)
Before product use	16.995 ± 4.625	-	-
Right after product use	15.543 ± 4.443	-8.562	0.000*

Interpretation of Table-1.

- Rate of change [a] (%) = [(after product use – before product use) / before product use] x 100
- Probability [b] (*p* value) *: $p<0.05$ by paired samples T-test

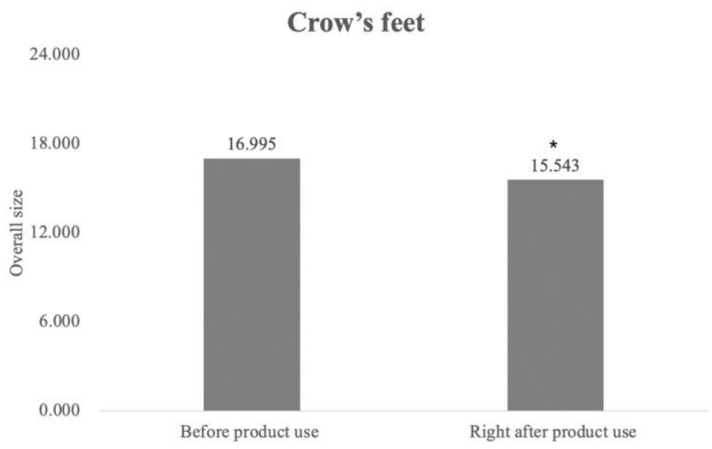

Figure 1. Results of crow's feet change using Antera 3D.

3.2. Measurement Results of Skin Moisture Content

Skin moisturization was measured before and right after using the test products. The Skin moisture content was 60.383 ± 8.466 A.U. before using the products, but right after using the products, it was increased to 70.756 ± 7.079 A.U. (Figure 2). Analyzing the rate of change by setting the before value at 100%, it increased by 17.988% right after the use of the products (Table 2). The measured value of skin moisture content statistically significantly increased right after using the test products compared to before using the products ($p<0.05$).

Table 2. Rate of change of skin moisture content.

Time	Average ± STD (A.U.)	Rate of change [a] (%)	Probability [b] (*p* value)
Before product use	60.383 ± 8.466	-	-
Right after product use	70.756 ± 7.079	17.988	0.000*

Interpretation of Table 2.

- Rate of change [a] (%) = [(after product use – before product use) / before product use] x 100
- Probability [b] (*p* value) *: $p<0.05$ by paired samples T-test

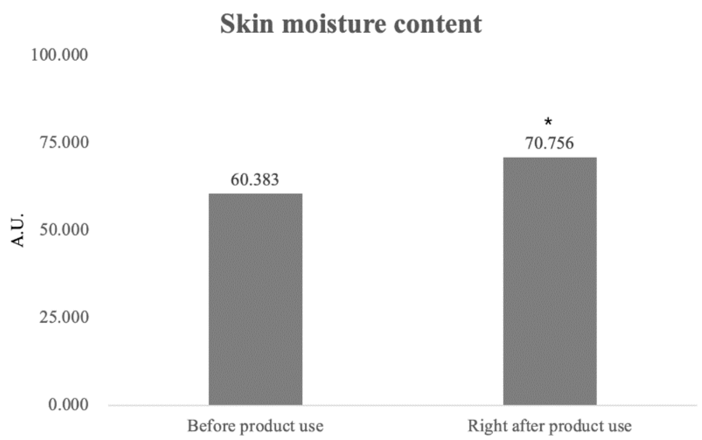

Figure 2. Results of skin moisture content change using Corneometer.

3.3. Measurement Results of TEWL

TEWL was measured before and right after using the test products. The TEWL was 14.048 ± 2.539 g/m^2h before using the products, but right after using the products, it was decreased to 11.614 ± 2.773 g/m^2h (Figure 3). Analyzing the rate of change by setting the before value at 100%, it decreased by 17.749% right after the use of the products (Table 3). Measured value of TEWL statistically significantly decreased right after using the test products compared to before using the products ($p<0.05$).

Table 3. Rate of change of TEWL.

Time	Average ± STD (g/m^2h)	Rate of change[a] (%)	Probability[b] (*p* value)
Before product use	14.048 ± 2.539	-	-
Right after product use	11.614 ± 2.773	-17.749	0.007*

Interpretation of Table 3.
- Rate of change [a] (%) = [(after product use – before product use) / before product use] x 100
- Probability [b] (*p* value) *: $p<0.05$ by paired samples T-test

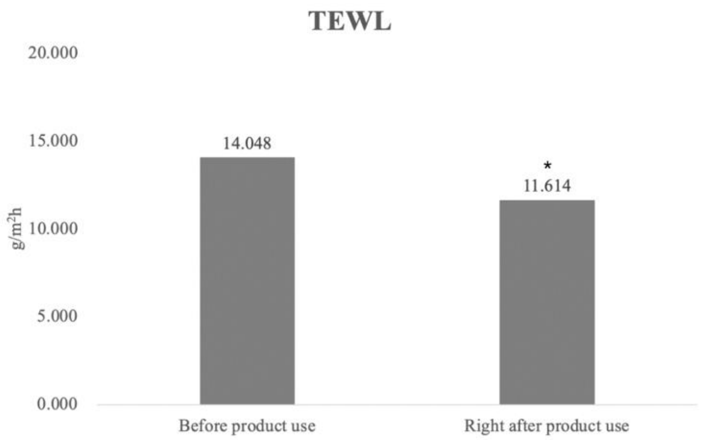

Figure 3. Results of TEWL using Vapometer.

3.4. Evaluation of Skin Adverse Reaction

There were no skin adverse events such as erythema, edema, scaling, itching, stinging, burning, tightness, pricking reported after using the test products (Table 4).

Table 4. Assessing skin adverse events.

Time	Erythema	Edema	Scaling	Itching
Right after product use	-	-	-	-
Time	**Stinging**	**Burning**	**Tightness**	**Pricking**
Right after product use	-	-	-	-

4. Conclusion

This study focused on proving that the antiaging cosmetics, which are four types of Biotoc Regen (Ampoule, Serum, Cream, and Feel) containing 1,000ppm of Pal-GHK-R4 have clinical effects on immediate wrinkle improvement. As a result of total of 21 subjects used the test products once on the face area; 1) the rate of change of crow's feet statistically significantly decreased by 8.562% right after using the products ($p<0.05$); 2) the rate of change of skin moisturization statistically significantly increased by 17.988% right after using the products

(p<0.05); 3) the rate of change of TEWL statistically significantly decreased by 17.749% right after using the products (p<0.05); 4) there were no skin adverse events reported after using the products. Therefore, the test products containing palmitoyl heptapeptide, Pal-GHK-R4, which combines wrinkle improving peptide with skin penetrating peptide to maximize the efficacy by increasing the permeability of GHK are considered to have immediate clinical effects of improving crow's feet, skin moisturization, and TEWL.

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