Research Article

Clinical Evaluation of Anti-Wrinkle Cosmetics containing Enhanced Solubilized System on Adenosine using Mixed Hydrotropic Agents

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Abstract: Adenosine is a typical active ingredient in wrinkle improvement functional cosmetics. However, adenosine has significant problems in producing formulations with sufficient bioavailability due to its low aqueous solubility. To overcome this problem, using niacinamide and vanillin mixed hydrotropic solubilization technique, which lower the minimum hydrotrope concentration (MHC) for adenosine, we developed cosmetics containing 2 % adenosine, and then clinical effect on wrinkle improvement and moisturizing of the cosmetics was evaluated. As a result of measuring crow's feet, skin moisture content, and transepidermal water loss (TEWL), 1) the improvement rate of crow's feet after 2 weeks and 4 weeks was 6.984 % and 10.609 %, respectively (p<0.05); 2) the improvement rate of the skin moisture content after 2 weeks and 4 weeks was 8.871 % and 15.576 %, respectively (p<0.05); 3) the improvement rate of the TEWL after 2 weeks and 4 weeks was 10.685 % and 9.498 %, respectively (p<0.05). In other words, we maximized the wrinkle improvement effect of adenosine by increasing the aqueous solubility using mixed hydrotropic agents.

Keywords: Adenosine, hydrotropic agents, clinical study, crow's feet, wrinkle improvement cosmetics

1. Introduction

The dermal extracellular matrix (ECM), mainly secreted by fibroblasts, is a dynamic and complex structure composed of an interlocking mesh of fibrous proteins (collagen and elastin fibres) and glycosaminoglycans (GAGs), providing skin strength and elasticity (**Frantz C. et al., 2010**; **Muiznieks & Keeley 2013**). Wrinkle development is associated with the breakdown of these ECM. The degradation of elastin, in conjunction with reduction in collagen, appears to be a major culprit of wrinkle formation by disrupting the interlocking mesh structure (**Shin J.W. et al., 2019**; **Ganceviciene R. et al., 2012**).

Adenosine is a typical active ingredient in wrinkle improvement functional cosmetics (**Yeo S. et al., 2021**; **Kim H.Y. et al., 2017**). Adenosine exerts its effect through the A2A receptor ($A_{2A}R$), one of the adenosine related receptors in cells. Activation of adenosine $A_{2A}R$ promotes fibrosis and collagen synthesis, leading to a decrease in wrinkles (**Perez-Aso M. et al., 2014**; **Valls M.D. et al., 2009**; **Borea P.A. et al., 2017**). Adenosine is considered as a safe material since it is a naturally occurring nucleoside which is present in various forms in all cells of the human body (**Belsito M.D. et al., 2019**). However, adenosine has significant problems in producing formulations with sufficient bioavailability because it is a sparingly soluble molecule whose solubility in water is lower than 8 mg/mL at room temperature (**Lee & Jin 2019**).

In order to overcome this problem, hydrotropy technique has been used to increase the aqueous solubility of various poorly water-soluble drugs by adding a large amount of second solutes (**Lee J. et al., 2003**). Hydrotropes are amphiphilic molecules and possess the ability to increase the aqueous solubility of sparingly soluble solutes, resulting in a several-fold increase of the solubility under normal conditions (**Kunz W. et al., 2016**; **Maheshwari R.K. et al., 2006**). Hydrotropes exhibit minimum hydrotrope concentration (MHC), a threshold concentration for solubilization (**Shimizu & Matubayasi 2014**). The agents which assist this phenomenon are called hydrotropic agents such as urea, sodium salicylate, vanillin, niacinamide, etc (**Maheshwari R.K. et al., 2010**; **Abraham S. et al., 2014**).

Previous studies on the clinical effect of adenosine-containing products in wrinkle improvement have been conducted in low content (**Abella M.L., 2006**; **Kang G. et al., 2018**). In this study, high content adenosine formulations were developed using niacinamide and vanillin mixed hydrotropic solubilization technique, which lower the MHC for adenosine. Then clinical effect on wrinkle improvement and moisturizing of cosmetics containing 2 % adenosine was evaluated by measuring crow's feet, skin moisture content, and transepidermal water loss (TEWL).

2. Materials and Methods

2.1. Clinical Study Protocol

Test products used in this clinical study are three types of cosmetics (skin softener, lotion, and cream) containing 2 % adenosine. Total 20 subjects who signed consent form voluntarily and satisfied with inclusion criteria and without exclusion criteria were selected. The selected subjects were women whose average age was 49.952 ± 4.985. The subjects used the test products for 4 weeks on a designated test area with crow's feet, then skin measurements were taken before, 2 weeks after, and 4 weeks after using the test products. Before measurements, subjects washed their test area with the same cleanser, followed by 30 min of stabilization. All the processes were conducted in an indoor environment where the temperature and humidity are constant and by the same conditions.

2.2. Crow's Feet Measurement

Crow's feet were measured by using a Antera 3D to photograph crow's feet. The photograph was analyzed and used as an evaluation material for crow's feet. The analyzed wrinkle variable is the overall size. Since the analyzed value and the improvement degree of wrinkle are inversely proportional, it means that the lower the analyzed value, the better the improvement of crow's feet.

2.3. Skin Moisture Content Measurement

Skin moisture content was measured by using a Corneometer CM 825. It was measured 3 times and averaged to evaluate the skin moisture content, and the unit is A.U. (Arbitrary Unit). The measured value is proportional to the skin moisture content, which means that the higher the measured value, the better the skin moisture content.

2.4. TEWL Measurement

TEWL was measured by using a Vapometer. The value measured once was used as the evaluation data of TEWL, and the unit is g/m^2h. Since the measured value and the improvement degree of TEWL are inversely proportional, it means that the lower the measured value, the better the improvement of TEWL.

2.5. Statistical Analysis

Statistical significance of changes was determined by using SPSS v23.0 (IBM, USA). If normality is accepted, paired samples T-test is used or otherwise Wilcoxon signed ranks test is used. A *p*-value of less than 0.05 is considered statistically significant.

3. Results and Discussions

3.1. Measurement Results of Crow's Feet

Using the optimal combination of niacinamide and vanillin, which lower the MHC for adenosine, cosmetics containing 2 % adenosine were developed. Crow's feet were measured before, 2 weeks after, and 4 weeks after using the test products to evaluate the wrinkle improvement effect. The overall size of crow's feet was 19.510 ± 7.889 before using the products. On the contrary, 2 weeks after and 4 weeks after using the products, it was gradually decreased to 17.940 ± 6.717 and 17.440 ± 7.266, respectively, and the rate of change was -6.984 % and -10.609 %, respectively. The analysis value of crow's feet revealed a statistically significant decrease 2 weeks after and 4 weeks after using the products compared to before using the products ($p<0.05$). The result is shown in Table 1 and Figure 1. In other words, adenosine, which is difficult to producing formulations with sufficient bioavailability due to its low aqueous solubility, was dissolved with hydrotropy technique, resulting in wrinkle improvement by 6.984 % after 2 weeks and 10.609 % after 4 weeks.

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	19.510 ± 7.889	-	-
2 weeks after product use	17.940 ± 6.717	-6.984	0.006†
4 weeks after product use	17.440 ± 7.266	-10.609	0.004†

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 Wilcoxon signed ranks test

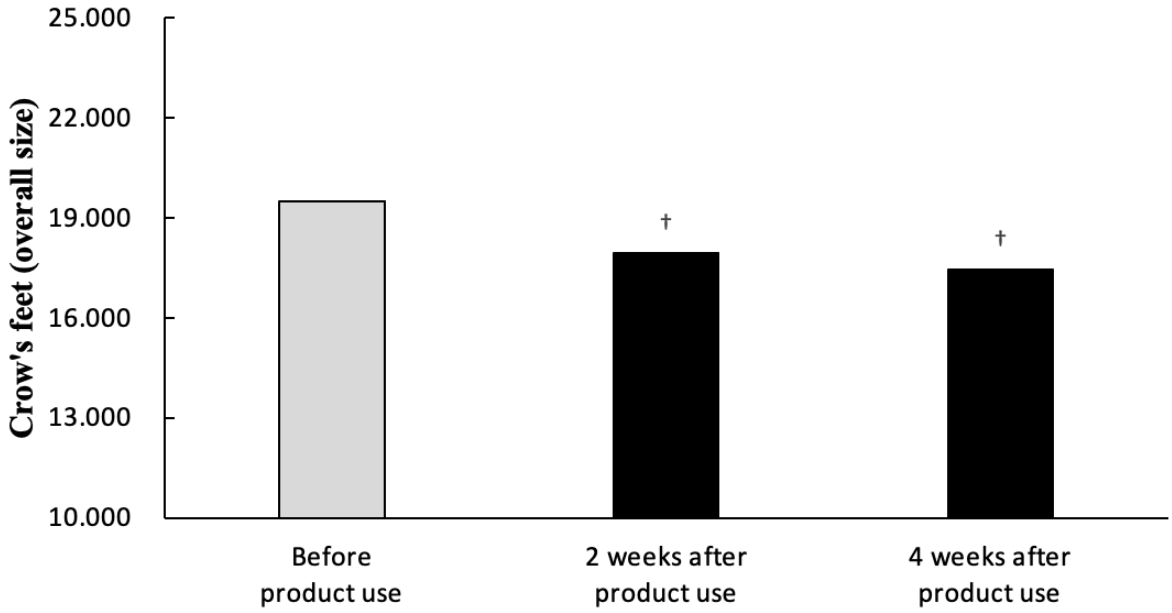

Figure 1. Results of crow's feet change using Antera 3D.

3.2. Measurement Results of Skin Moisture Content

Moisturizing of skin is crucial for healthy skin function and moisturizers are essential components of cosmetics (**Purnamawati S. et al., 2017**; **Lodén M., 2003**). Skin moisture content was measured to evaluate the moisturizing effect of the test products. The skin moisture content was gradually increased to 69.022 ± 6.582 A.U. and 73.235 ± 7.860 A.U. 2 weeks after and 4 weeks after using the products while it was 63.677 ± 7.489 A.U. before using the products (Figure 2). The rate of change after 2 weeks and 4 weeks was 8.871 % and 15.576 %, respectively (Table 2). The change of skin moisture content revealed a statistically significant increase (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	63.677 ± 7.489	-	-
2 weeks after product use	69.022 ± 6.582	8.871	0.000*
4 weeks after product use	73.235 ± 7.860	15.576	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

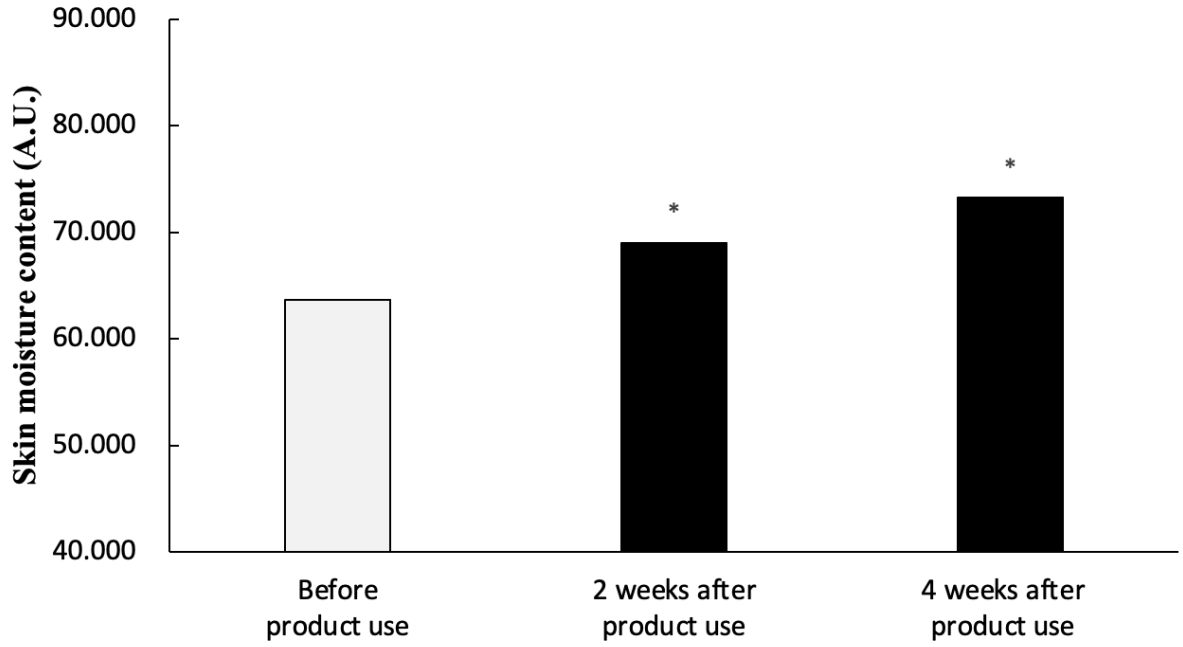

Figure 2. Results of skin moisture content change using Corneometer.

3.3. Measurement Results of TEWL

TEWL was measured to evaluate the moisturizing effect of the test products. The TEWL was decreased to 12.690 ± 3.424 g/m^{2}h and 13.035 ± 4.297 g/m^{2}h 2 weeks after and 4 weeks after using the products while it was 14.420 ± 3.899 g/m^{2}h before using the products (Figure 3). The rate of change after 2 weeks and 4 weeks was -10.685 % and -9.498 %, respectively (Table 3). The change of TEWL revealed a statistically significant decrease (p<0.05). It was concluded that moisturizers in the test products improves skin moisture content by providing water to the skin from their water phase and reduces TEWL by increasing occlusion from their oil phase (**Purnamawati S. et al., 2017**; **Lodén M., 2003**).

Table 3. Rate of change of TEWL.

Time	Average ± STD (g/m^{2}h)	Rate of change [a] (%)	Probability [b] (p value)
Before product use	14.420 ± 3.899	-	-
2 weeks after product use	12.690 ± 3.424	-10.685	0.007*
4 weeks after product use	13.035 ± 4.297	-9.498	0.044*

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

3.4. Evaluation of Skin Adverse Effects

To ensure the safety of the cosmetics containing 2 % adenosine, skin adverse effects, e.g., erythema, edema, scaling, itching, stinging, burning, tightness, pricking were assessed. There were no skin adverse effects during the test period (Table 4). Therefore, we confirmed the safety as well as the efficacy of the test products.

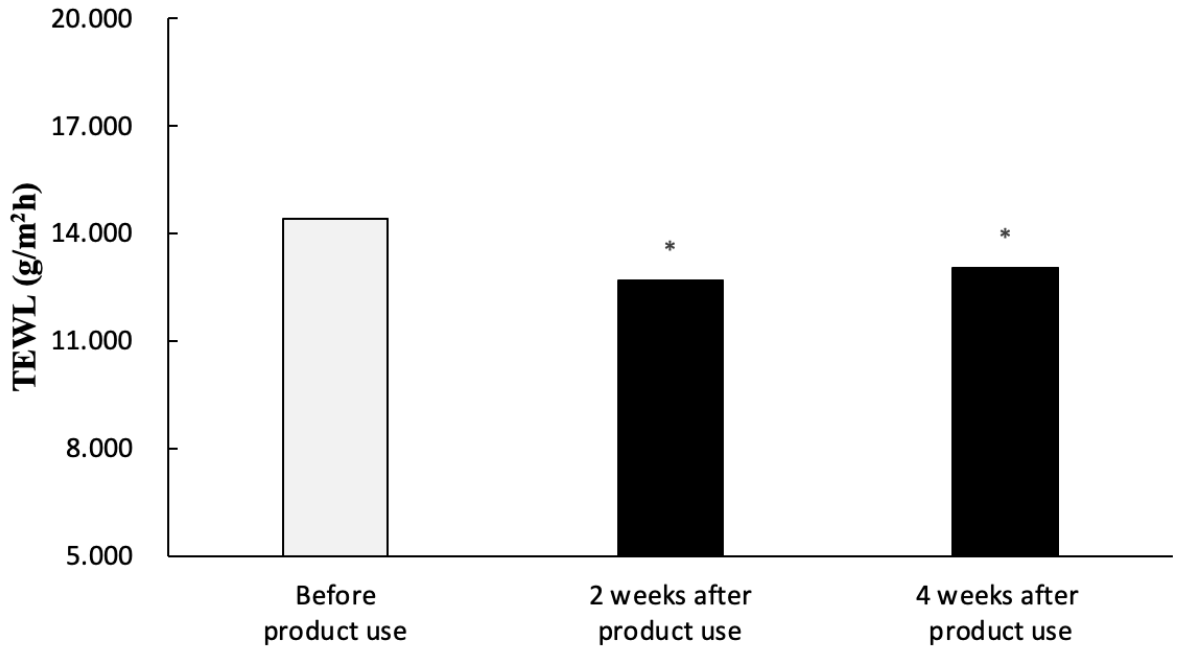

Figure 3. Results of TEWL using Vapometer.

Table 4. Assessing skin adverse effects.

Time	Erythema	Edema	Scaling	Itching
2 weeks after product use	-	-	-	-
4 weeks after product use	-	-	-	-
Time	**Stinging**	**Burning**	**Tightness**	**Pricking**
2 weeks after product use	-	-	-	-
4 weeks after product use	-	-	-	-

4. Conclusion

This study aimed to maximize the wrinkle improvement effect of adenosine by increasing the aqueous solubility using hydrotropic agents. In this study, we established the optimal combination of niacinamide and vanillin to lower the MHC for adenosine. Using this hydrotropic solubilization technique, three types of cosmetics (skin softener, lotion, and cream) containing 2 % adenosine were developed. Then clinical effect on wrinkle improvement and moisturizing of the test products was evaluated by measuring crow's feet, skin moisture content, and TEWL. The improvement rate of crow's feet after 2 weeks and 4 weeks was 6.984 % and 10.609 %, respectively (p<0.05). The improvement rate of the skin moisture content after 2 weeks and 4 weeks was 8.871 % and 15.576 %, respectively (p<0.05) and the improvement rate of the TEWL after 2 weeks and 4 weeks was 10.685 % and 9.498 %, respectively (p<0.05). In this study, we developed high content adenosine cosmetics with sufficient bioavailability using mixed hydrotropic agents and proved their significant wrinkle improvement effect through the clinical trials. Therefore, based on this study, we intend to contribute to the development of functional cosmetics for wrinkle improvement.

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