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Itching and Atopy Relief using Azulene Derivatives and High-Content Ceramide Skin Barrier Nano-Liposome Structures by Dry Skin

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Abstract- Atopic dermatitis (AD), a chronic inflammatory skin disease, has increased in industrialized nations, impacting approximately 15 to 20% of children and 1 to 3% of adults worldwide. Previous studies suggest that the impairment of the skin barrier and immunological dysfunction can be considered as the two main factors involved in AD. In this study, a balm formulation containing 0.02% guaiazulene, an azulene derivative, and 1.0% high-content ceramide skin barrier nano-liposome structure was developed to effectively treat AD by normalizing these main factors, and clinical trials were conducted with 23 subjects. Compared to before using the product, (1) the skin moisture content in the forearm area increased significantly ($p<0.05$) by 25.20% and 63.61% after 2 weeks and 4 weeks of use of the product, respectively, (2) the amount of skin moisture loss in the forearm area decreased significantly ($p<0.05$) by 12.04% and 17.79% after using the product for 2 weeks and 4 weeks, respectively, (3) the skin texture (roughness) of the forearm area significantly decreased ($p<0.05$) by 3.00% and 4.18% after 2 weeks and 4 weeks of use, respectively, (4) the degree of itchiness due to dry skin significantly decreased ($p<0.05$) by 47.91% after 2 weeks of use and 69.71% after 4 weeks of use, and the degree of sleep disturbance due to itching caused by dry skin significantly decreased ($p<0.05$) by 46.22% after using the product for 2 weeks and by 74.07% after using the product for 4 weeks. This study confirmed the outstanding clinical effects of the formulation containing guaiazulene and ceramide on itching relief caused by dry skin.

Keywords: *Clinical study, Itching relief, Guaiazulene, Ceramide, Cosmetics*

1. Introduction

Atopic dermatitis (AD), which is a multifactorial chronic inflammatory skin disease, is rising in industrialized nations [1]. The characteristic of patients with AD is that they have dry skin and go through systemic itching. It can lead to disrupt sleep, cause emotional distress, and lower quality of life by scratching their skin [2].

The pathogenesis of AD is not currently clear, but there have been two main theories; inside-out theory and outside-in theory. Inside-out theory is that inflammatory reaction precedes AD. Immunological abnormalities produce various inflammatory mediators such as intereukin-4 (IL-4), IL-13 and thymic stromal lymphopoietin (TSLP) etc. and consequently lead to skin barrier dysfunction. Outside-in theory is that skin barrier abnormalities precede to AD. Impaired skin barrier could be accompanied by increased trans epidermal permeation of allergens and be observed increased *Staphylococcus aureus* (*S. aureus*) colonies, and impaired skin barrier leads to cutaneous inflammation, consequently. According to claims related to inside-out theory, it has been reported recently that filaggrin (FLG) expression downregulation is associated with AD [3-6]. But the most important thing about AD is that it is examined that both inflammation reaction and breakdown of skin barrier occur at the same time in the lesion of patients with AD. That is, it is critical to alleviate and restore both inflammation in skin and skin barrier dysfunction normally in effectively treating AD.

Park et al. reported that guaiazulene plays a role in downregulating inflammatory cytokines and curtailing

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immunological abnormalities [7]. Moreover, it has demonstrated that guaiazulene exerts anti-oxidant activity, anti-microbial activity, balancing the hydration of epidermal and dermatitis in vitro and in vivo [8-9]. Ceramide, which accounts for 50% of intercellular lipids, helps to keep human skin barrier healthy. In other words, it has the effect on corneocytes adhesion-deadhesion, tumor cell apoptosis and skin barrier function [10]. However, it has been reported that ceramide, especially ceramide NP, is reduced in the *stratum corneum* (SC) in patients with AD [11]. Also, ceramide degradation produces sphingosine by ceramidase, which has a great effect on anti-microbial activity against *S. aureus* [12-13]. In addition, *S. aureus* infection is observed over 90% of skin lesions of AD patients [14].

In some previous studies, each guaiazulene and ceramide NP turned out to be effective agents in improving abnormal skin. However, it has not been studied in a formulation containing guaiazulene that regulates inflammation reaction and ceramide NP that improves disrupted skin barrier. Here, we developed a balm formulation containing guaiazulene and ceramide NP. We conducted clinical research to see if the formulation can be used for itching relief for those who has dry skin in this study. Specifically, we assessed the degree of skin moisture content, the degree of trans-epidermal water loss (TEWL), the degree of improvement of skin texture (roughness), visual analogue scale (VAS) and safety of the test formulation for 4 weeks.

2. Materials and Methods

2.1 Purpose of clinical study

The primary objective is to demonstrate itching resulted from dry skin is relieved temporally through clinical research (*in vivo*) with the test formulation for 4 weeks. The secondary objective is to evaluate skin adverse event (safety) of the test formulation containing 0.02% guaiazulene and 1.0% high-content ceramide skin barrier nano-liposome structure.

2.2 Clinical trials protocol for a balm formulation

Total 23 subjects are satisfied with inclusion and with no exclusion criteria and signed consent form. Eligible participants are women aged 20-60 years. The average age of the participants selected as subjects in this study was 47.3 ± 10.6. All Subjects are women with dry skin whose skin moisture content was less than 29 arbitrary unit (A.U.) when their forearm area was measured by Corneometer® CM 825. Test formulation is a balm formulation like lip balm. We instructed them to apply the test formulation to their forearm area whenever they feel itchy frequently. All evaluations were measured three times after subjects washed their forearm area personally with the same cleanser and were acclimatized for 30 minutes under a constant temperature (22 ± 2℃) and humidity (50 ± 5%). All measurements were taken three times each before, 2 weeks after test formulation use, and 4 weeks after test formulation use, and averaged. Rate of change was calculated as follows:

Rate of change (%) =[(measurement value after application - measurement value before application)/ measurement value before application] × 100

2.3 Measurement method

Skin Moisture Content Assessment. Corneometer® CM 825 (Courage and Khazaka, Cologne, Germany) was used for measuring skin moisture content. We measured three times at each before application, 2 weeks after application, 4 weeks after application, and represent mean and standard deviation (SD). It is used to evaluate hydration level in SC. The measurement value is expressed as A.U.. The capacitance changes according to skin water content, and the measurement value by using Corneometer ® CM 825 is proportional to skin moisture content in SC. That is, the higher the measured value is, the more hydrated the SC is.

TEWL Assessment. Tewameter® TM300 (Courage and Khazaka, Cologne, Germany) was used for measuring TEWL. The measurement value is expressed as is g/hm^2. The Tewameter is based on the water diffusion principle from SC [15]. Using this principle, TEWL is calculated from the increasing relative humidity (RH) in the probe. The TEWL reading from the probe starts after the probe is stabilized 30-60 seconds [16]. TEWL is inversely proportional to epidermal thickness, especially SC [17-18].

Skin Texture (Roughness) Assessment. Antera 3D® CS (Miravex Limited, Ireland) was used for measuring the change of skin texture (roughness). This instrument can analyze various parameter related to skin texture, wrinkle and pigmentation, and captures the skin surface at high resolution and converts the captures into 3-dimension image. The converted image is analyzed and quantified changes in skin condition via an internal algorithm. Ra, mean roughness, was calculated. The lower Ra, the better improvement of skin texture (roughness).

VAS Assessment. Subjective evaluation of itching caused by dry skin and sleep disturbance due to itching was assessed using VAS. The VAS evaluation is one of the pain scales that evaluates the intensity of subjective pain (0 = not itchy at all; 10 = incredibly itchy; 0 = not sleep disturbance at all; 10 = extremely sleep disturbance). On the three days leading up to the time points (before using the test formulation, 2 weeks after using the test formulation, and 4 weeks after using the test formulation), data was collected (D-3, D-2 and D-1). The values of itchiness and sleep disturbance that subjects assess subjectively were averaged and used as the evaluation index for the improvement of itchiness and sleep disorder.

2.4 Assessment of safety

Researcher observed and evaluated the test area (forearm) of the subjects, and recorded the condition of the test area through interview with the participants. Our clinical trials protocol is that when cutaneous adverse reactions due to the formulation occurs, researchers fill the adverse reaction report in. The relevance to the test formulation is judged by the principal researcher.

2.5 Statistical analysis

All data from measurements were analyzed using the Statistical Product and Service Solution (SPSS) Package Program ver. 27 (IBM, USA). Testing normality of the data collected were analyzed using Shapiro-Wilk test and Kurtosis & Skewness. For comparison before and after the test formulation, paired t-test was used when normality was satisfied by performing a normality test, and Wilcoxon Signed-Ranks test was used when normality was not satisfied. If the *p*-value is less than 0.05 ($p<0.05$), it is considered statistically significant.

3. Results and Discussion

3.1 Increased skin moisture content

Table 1 shows the results of three repeated measurements of skin moisture content. Before application, the water content of SC in the forearm was 26.04 ± 2.14 A.U.. 2 weeks after application, the skin moisture content was 32.54 ± 2.71 A.U., and 4 weeks after application skin moisture content was 42.49 ± 3.97 A.U.. As a result, 2 weeks after application and 4 weeks after application increased statistically significantly compared to before application ($p<0.000$). We examined the rate of change after 2 weeks and after 4 weeks increased by 25.20% and 63.61%, respectively. It was confirmed that the skin moisture content increased as the period of use prolonged. Thus, the use of the balm formulation replenished dry skin with water.

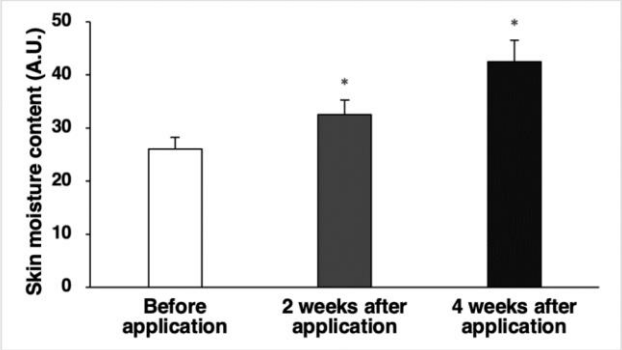

Figure 1- Change of skin moisture content after using the test formulation (n = 23). *****$p<0.05$ versus before application.

Table 1. Result of skin moisture content measurement

Time point	Mean (A.U.)	SD (A.U.)	Rate of change (%)	*p*-value
Before application	26.04	2.14	-	-
2 weeks after application	32.54	2.71	25.20	0.000*
4 weeks after application	42.49	3.97	63.61	0.000*

p-value (Probability)*: $p<0.05$ by Paired samples T-test

3.2 Reduction of TEWL

Table 2 shows the result of three repeated measurements of TEWL. Before application, TEWL of SC in the forearm was 8.17 ± 1.15 g/hm^2. 2 weeks after application, TEWL was 7.17 ± 1.37 g/hm^2, and 4 weeks after application TEWL was 6.64 ± 1.04 g/hm^2. As a result, 2 weeks after application and 4 weeks after application decreased statistically significantly compared to before application ($p<0.002$). We examined the rate of change after 2 weeks and 4 weeks decreased by 12.04% and 17.79%, respectively. It was confirmed that TEWL decreased as the period of use prolonged. Patients with AD tend to have the feature of thin stratum corneum. Thus, TEWL that is inversely proportional to the thickness of SC increase. As a result of clinical trials with the balm formulation, it was confirmed that TEWL decreased, and we considered that the thickness of the SC, which was thin, increased based on clinical research's results, thereby restoring impaired skin barrier [19].

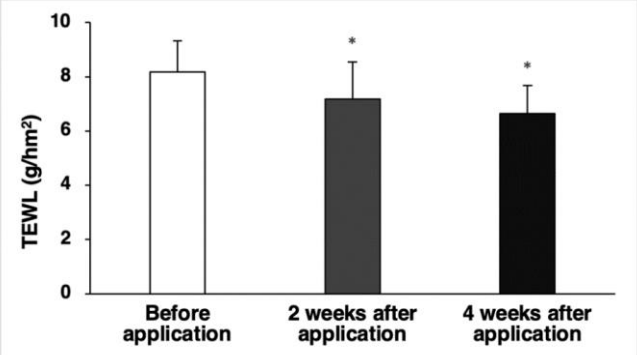

Figure 2- Change of TEWL after using the test formulation (n = 23). *****$p<0.05$ versus before application.

Table 2. Result of TEWL measurement

Time point	Mean (g/hm^2)	SD (g/hm^2)	Rate of change (%)	*p*-value
Before application	8.17	1.15		
2 weeks after application	7.17	1.37	-12.04	0.002*
4 weeks after application	6.64	1.04	-17.79	0.000*

p-value (Probability)*: $p<0.05$ by Paired samples T-test

3.3 Evaluation of skin texture (roughness)

Table 3 shows the result of three repeated measurements of skin texture. Before application, skin texture of epidermis in the forearm was 5.37 ± 1.07 Ra. 2 weeks after application, skin texture was 5.19 ± 1.00 Ra, and 4 weeks after application skin texture was 5.13 ± 0.97 Ra. As a result, 2 weeks after application and 4 weeks after application decreased statistically significantly compared to before application ($p<0.011$). We examined the rate of change after 2 weeks and 4 weeks decreased by 3.00% and 4.18%, respectively. It was confirmed that roughness of skin decreased as the period of use prolonged.

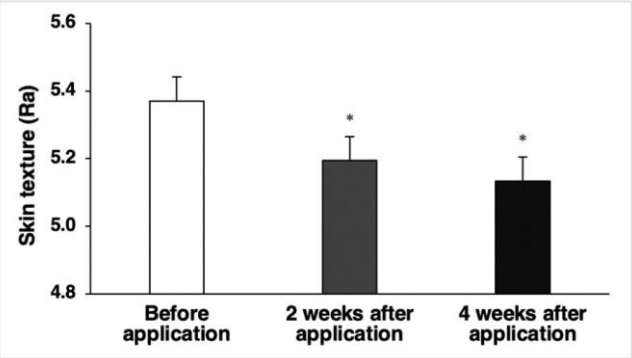

Figure 3- Change of skin texture after using the test formulation (n = 23). *****$p<0.05$ versus before application.

Table 3. Result of skin texture measurement

Time point	Mean (Ra)	SD (Ra)	Rate of change (%)	*p*-value
Before application	5.37	1.07	-	-
2 weeks after application	5.19	1.00	-3.00	0.011*
4 weeks after application	5.13	0.97	-4.18	0.000*

p-value (Probability)*: $p<0.05$ by Paired samples T-test

3.4 Improvement of itching and sleep disturbance

Systemic pruritus affects the difficulty falling asleep and reducing sleep efficiency by scratching. That is, sleep abnormalities has a negative effect on quality of life in children and adults with AD [20].

Before application, the degree of itching in the forearm was 6.70 ± 1.49. 2 weeks after application, the degree of itching in the forearm was 2.00 ± 1.38, and 4 weeks after application, the degree of itching was 2.00 ± 1.38. As a result, 2 weeks after application and 4 weeks after application showed statistically significantly greater compared to before application (Figure 4, Table 4). We examined the rate of change after two weeks and four weeks decreased by 47.91% and 69.71%, respectively. According to the decreasing trend of the degree of itching, before application, the degree of sleep disturbance due to itching caused by dry skin was 5.83 ± 1.67. 2 weeks after application, the degree of sleep disturbance was 3.22 ± 1.54, and 4 weeks after application, the degree of sleep disturbance was 1.43 ± 1.27. As a result, after 2 weeks of application and after 4 weeks of application, sleep disturbance decreased statistically significantly compared to before application ($p<0.000$). We examined the rate of change after 2 weeks and 4 weeks decreased by 46.22% and 74.07%, respectively. It was confirmed that the degree of itching and sleep disturbance decreased as the period of use of the test formulation prolonged. We presumed that guaiazulene included in the preparation regulated inflammatory response, and ceramide NP was replenished to impaired skin and it is degraded by ceramidase and produced fatty acids and sphingosine [21]. Sphingosine has the great effect on anti-bacterial activity against *S. aureus*. Due to the decline of *S. aureus* colonies in epidermidis, superantigens were curtailed. Ultimately, we considered guaiazulene and ceramide NP created a synergy effect, thereby reducing itching and sleep disturbance.

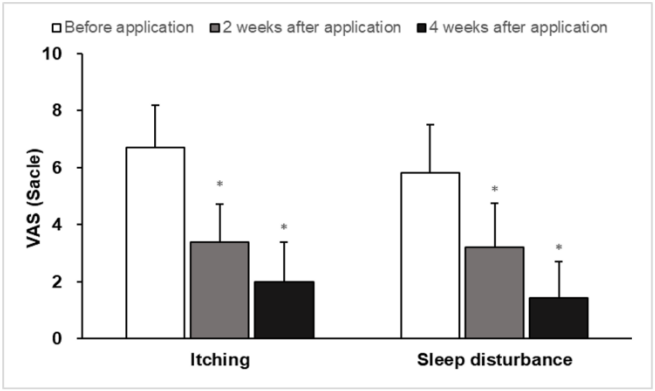

Figure 4- Change of the degree of itching and sleep disturbance (n = 23). ***p<0.05 versus before application.

Table 4. Improvement of itching and sleep disturbance

Symptom	Time point	Mean (Scale)	SD (Scale)	Rate of change (%)	p-value
Itching	Before application	6.70	5.83	-	-
	2 weeks after application	3.39	3.22	-47.91	0.000*
	4 weeks after application	2.00	1.43	-69.71	0.000*
Sleep disturbance	Before application	1.49	1.67	-	-
	2 weeks after application	1.34	1.54	-46.22	0.000*
	4 weeks after application	1.38	1.27	-74.07	0.000*

p-value (Probability)*: p<0.05 by Paired samples T-test

3.5 Evaluation of safety

There weren't any skin adverse events after using the test formulation during the clinical study period. Any side effect on skin such itching, stinging, burning, pricking, tightness, erythema, edema, scaling, papule was not observed in a total of 23 subjects. The balm preparation provoked no irritation and is suitable for dry skin.

Table 5. Assessment of skin adverse events

Skin adverse events	2 weeks after application	4 weeks after application
Itching	-	-
Stinging	-	-
Burning	-	-
Pricking	-	-
Tightness	-	-
Erythema	-	-
Edema	-	-
Scaling	-	-

4. Conclusion

The basic feature of AD are low water content in SC, high TEWL, xerosis and pruritus. As a result, after clinical trials was conducted with the balm formulation on those who have dry skin for 4 weeks, we confirmed the increased hydration level, the decreased TEWL and itching relief, thereby improving sleep disturbance of dry skin. These changes were statistically significant (p<0.05). Taken together, these results in this study implied that the balm formulation including 0.02% guaiazulene and 1.0% ceramide NP can help to restore breakdown of skin barrier and temporarily relieve itching in patients with AD. It was confirmed that sleep disorders that patients with AD experienced can be improved. We considered it to be used for the agent, which is immunological modifier and skin barrier repairer. Therefore, we concluded that the preparation containing 0.02% guaiazulene and 1.0% ceramide NP can be usefully used for improving the quality of life in patients with AD.

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