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Clinical Efficacy and Safety of an Anti-Acne Cosmetics Containing Natural Salicylic Acid and an Epidermal Penetrating Peptide

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Abstract
The purpose of this study is to investigate clinical efficacy and safety on anti-acne cosmetics containing 0.5% natural salicylic acid and 0.01% transdermal penetrating arginine peptide (R6). In the case of non-inflammatory lesions, the results showed that the lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks. Statistically, non-inflammatory lesions decreased statistically after 4 weeks and after 6 weeks except for 2 weeks of use compared to before using acne cream ($p<0.02$). For inflammatory lesions, the results represented that the total lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks. For inflammatory lesions, the inflammatory variable decreased statistically, both after 2 weeks of use, 4 weeks after use and 6 weeks after use ($p<0.005$). For total lesions (non-inflammatory lesions + inflammatory lesions), The results showed that the total lesions improved from 28.96 ± 24.25 after 2 weeks to 43.68 ± 17.97 after 4 weeks and to 51.41 ± 18.19 after 6 weeks. For total lesions, the total lesions decreased statistically, both after two weeks of use, four weeks after use and six weeks after use ($p<0.001$). No specific skin adverse events were observed in all subjects participating that participated in the present study. Therefore, the test product containing a natural salicylic acid and a skin penetrating peptide is have beneficial effects in reducing acne for 6 weeks.

Keywords*: Anti-acne, Efficacy, Safety, Natural Salicylic acid, Transdermal Penetrating Peptide.*

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1. INTRODUCTION

Acne vulgaris is a chronic inflammatory disease caused by the complex influences of abnormal lipid metabolism (endocrine factors), abnormal keratinization and bacterial proliferation. It affects pilosebaceous units on the face, chest and back during and after puberty. Acne is a skin disorder that suppresses an individual's self-esteem about physical appearance and has a clinical onset during puberty and adolescence [1-2]. The pathogenesis of acne is regulated by sebum hypersecretion in deformed follicles, which leads to microcomedones, and the follicular hyperproliferation of microcomedones causes inflammation [3], and comedones [4] in both open and closed types appearing in papules, pustules, nodules and cysts [5]. The resulting skin condition with sebum enrichment is prone to the anaerobic growth of *Propionibacterium acnes*, which is the main causative microorganism in acne. In addition, *Staphylococcus epidermidis* and *Pitryosporum ovale* are present in acne lesions [6]. Proliferation of these microorganisms, mainly *P. acnes*, leads to inflammatory lesions and severe acne.

The main causes of acne are increased sebum secretion due to the imbalance of hormones in the body, clogged pores by excessive sebum secretion by androgen, male hormone, and decomposed pores into the cause of acne to produce fatty acids and cause inflammation of the fatty acids. In normal skin, the sebum is released through the hair follicles, but if the sebum is not released from the skin and cannot leave the area around the follicle, inflammatory bacteria are reproduced, which causes acne. Also, it is caused by excessive stress, lack of sleep, and poor cleaning of oil-rich cosmetics. It is also caused by adrenal cortex hormones that stimulate the sebum. Traditional medicines for acne are treated with salicylic acid, benzyl peroxides, antibiotics, retinoids and oral contraceptives. Therefore, acne formation needs to be addressed, particularly acne vulgaris. In addition to adolescent acne, drugs are a relatively common cause of eruptions resembling acne. Drug-induced acne or acneiform dermatoses that can have a sudden onset e.g. within 1 day of drug administration can be resolved after the drug is stopped. Acneiform dermatoses have an unusual lesion distribution, such as inflammatory papules and pustules that are small and uniform in size (monomorphic) and can lead to secondary comedones of which the earliest histological event is spongiosis followed by lymphocytic and neutrophilic infiltrates, respectively [7]. Those drugs capable of producing eruptions have been summarized elsewhere [8]. Therefore, although the initial causes are different, the pathogenesis of acne vulgaris can be similar. Thus, some treatments are used for both adolescent and drug-induced acne. In this review, the therapeutic ingredients in topical applications relevant to the pathogenesis of acne vulgaris lesions were evaluated, as topical application is more feasible [9], especially with the naturally derived compounds already in use and candidate compounds.

Salicylic acid, a mild keratolytic and anti-inflammatory agent [10] was used to remove follicular clog [11] in various formulations, particularly an alcoholic solution for cleansing. This formulation posed better efficacy than benzoyl peroxide [12]. Salicylic acid is a milder agent compared with retinoids. A combination of salicylic acid, and benzoyl peroxide would increase treatment efficacy as their mechanisms are differ [13]. In addition to be a cleansing product, skin peeling using salicylic acid was found to significantly reduce comedones [14].

Recently, several studies have represented that cell-penetrating peptides (CPPs) can enhance the transdermal delivery of biomaterials [15-17]. Short arginine oligomer peptides enabled transport across the epidermis, when applied topically to either mouse or human skin [18]. It was represented by the present author [19] that high concentration of caffeine aqueous solutions could be successfully prepared, and transdermal penetration was improved with a short arginine oligomer (R6). In this study, we examined the clinical efficacy and safety on anti-acne cosmetics containing 0.5% natural salicylic acid and 0.01% transdermal penetrating arginine peptide (R6).

2. MATERIAL AND METHODS

2.1 Test formulation

The main ingredients of test formulation 0.5% natural salicylic acid and 0.01% R6 as a skin penetrating peptide (arginine oligomer peptide). The other ingredients contained emulsifier, oil, humectants, fragrance and deionized water in test products. Natural salicylic acid was purchased from Alban Muller Industrie in France and have a purity of at least 99.0%, and R6 was obtained from Dermafirm Co., Ltd, in Korea and have a purity of at least 98.0%.

2.2 Study Protocol

In this study, KC Skin Research Center conducted the body efficacy evaluation according to the tenets of the Declaration of Helsinki and complied with the Guideline of Bioethics and Safety Act by the Ministry of Health and Welfare. The study was approved by the Institutional Review Board of KC Skin Research Center Co., Ltd., in Korea (KC-IRB-029).

1) Criteria for selection of subjects

This study selected voluntarily participating healthy men and women who are 15 years old or older and who can be classified as secondary or lower acne patients, who signed their participation by their own discretion.

2) Criteria for excluding the selection of subjects

We excluded women who were received an oral dose of retinoid preparation within six months of the study, received oral antibiotic therapy within one month of the start of the study, other local acne treatment within two weeks of the study, and the person or researcher determined that the clinical study could not be continued due to adverse effects during use.

3) Verification of the homogeneity of the subjects

The subjects who satisfied the criteria of selecting the subjects and those who did not have the criteria for selection exclusion were selected by homogeneity test for those who have relatively similar life environment, skin condition, skin care and cosmetics use.

4) Human body test design

The human body test was conducted for a total of 6 weeks

2.3 Evaluation Method

The clinical evaluation was conducted once every week before and after the start of the study and up to six weeks. Each visit was evaluated by the same researcher on clinical indicators, and the first and last visit was conducted with a digital camera. To check the frequency and extent of side effects, the subjects recorded the symptoms of the complaint at each visit. Objective indicators of clinical evaluation were compared with each visit by counting the number of closed and open blisters (non-inflammatory lesions) and agar (inflammatory lesions) throughout the facial region by visual observation of the same observer.

Subjective indicators of acne improvement may be divided into the following;

3: Marked improvement
2: Slight improvement
1: No change
0: Poor

The subjective indicators of the observer can be divided and expressed as follows by evaluating the clinical picture obtained by two observers before and after treatment.

3: Marked improvement
2: Slight improvement
1: No change
0: Poor

Side effects recorded the symptoms of the patient's complaint during the visit. The statistical process compared the results of two weeks, four weeks and six weeks based on 0 weeks, with the total number of lesions, non-inflammatory lesions and inflammatory lesions counted for all objective data. The statistics were based on the study t-test. The statistical significance of the data was $p<0.05$.

3. RESULTS AND DISCUSSION

At the beginning of the study, the target was set at 18, but the final evaluation was carried out with 16 members after two were dropped out for six weeks. Both were dropped out due to personal reasons, not to special side effects. The participants totaled 16, with four men, 14 women and an average age of 24.1.

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Objective Clinical evaluation calculated the improvement rate of lesions in each state based on the initial number of lesions.

For total lesions (non-inflammatory lesions + inflammatory lesions), the lesions were measured 3 times; after 2 weeks, after 4 weeks, and after 6 weeks. The results showed that the total lesions improved from 28.96 ± 24.25 after 2 weeks to 43.68 ± 17.97 after 4 weeks and to 51.41 ± 18.19 after 6 weeks (Table 1). For total lesions (non-inflammatory lesions + inflammatory lesions), the total lesions decreased statistically, both after two weeks of use, four weeks after use and six weeks after use ($p<0.001$).

Table 1: Results of total lesions (non-inflammatory lesions + inflammatory lesions) improvement rate at each observation point

Time	Improvement rate[a] ± STD (%)	Probability[b] (*p* value)
After 2 weeks	28.96 ± 24.25	0.05
After 4 weeks	43.68 ± 17.97	0.001 **
After 6 weeks	51.41 ± 18.19	0.001 **

• Improvement rate[a] (%) = [(# of initial lesions – # of lesion after use)

/ [# of initial lesions] x 100

• Probability[b] (*p value*) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

In the case of non-inflammatory lesions, five patients with less than two initial lesions could cause distortion of the results and were excluded from the statistical process and analyzed. The results represented that the total lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks (Table 2). Statistically, non-inflammatory lesions decreased statistically after 4 weeks and after 6 weeks except for 2 weeks of use compared to before using acne cream ($p<0.02$).

Table 2: Results of non-inflammatory lesions improvement rate at each observation point

Time	Improvement rate[a] ± STD (%)	Probability[b] (*p* value)
After 2 weeks	19.08 ± 35.46	0.05
After 4 weeks	40.00 ± 32.33	0.087 **
After 6 weeks	43.31 ± 28.49	0.001 **

• Improvement rate[a] (%) = [(# of initial lesions – # of lesion after use)

/ [# of initial lesions] x 100

• Probability[b] (*p value*) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

For inflammatory lesions, the results represented that the total lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks (Table 3). For inflammatory lesions, the inflammatory variable decreased statistically, both after 2 weeks of use, 4 weeks after use and 6 weeks after use ($p<0.005$) [20-22].

Table 3: Results of non-inflammatory lesions improvement rate at each observation point

Time	Improvement rate[a] ± STD (%)	Probability[b] (*p* value)
After 2 weeks	31.23 ± 35.13	0.05
After 4 weeks	47.40 ± 32.46	0.005 **
After 6 weeks	57.82 ± 26.54	0.001 **

• Improvement rate[a] (%) = [(# of initial lesions – # of lesion after use)

/ [# of initial lesions] x 100

• Probability[b] (*p value*) **: $p<0.05$ by Repeated measured ANOVA, post hoc Bonferroni correction

Subjective evaluations included 2 participants (12.5%) who felt that the subjects themselves improved significantly after the end of the study, 9 participants (56.25%) who felt slightly improved, and 5 participants (31.25%) who felt normal. None of the participants felt slightly worse or much worse.

For subjective evaluations of observers, evaluating clinical photographs obtained from participants at the start and end of the study by dermatologists, 4 participants (25%) showed significant improvement, 10 participants (62.5%) showed moderate improvement and 3 participants (18.8%) showed normal. None of the participants appeared to be slightly worse or worse.

In the test subjects, the presence of adverse skin reactions such as erythema, edema, scaling, itching, stinging, burning, tightness, ting (rickets, swelling, scurvy, itching, aching, burning, stiffness, tingling) among others was investigated every time subject presented themselves for analysis. No specific skin adverse events were observed in all subjects participating that participated in the present study (Table 4).

Table 4: Assessing skin adverse events

Time	Erythema	Edema	Scaling	Itching
After 2 weeks	-	-	-	-
After 4 weeks	-	-	-	-
After 6 weeks	-	-	-	-

Time	Stinging	Burning	Tightness	Prickling
After 2 weeks	-	-	-	-
After 4 weeks	-	-	-	-
After 6 weeks	-	-	-	-

Step=1: Weak, 2: Medium, 3: Severe

4. CONCLUSION

In this study, we examined the clinical efficacy and safety on anti-acne cosmetics containing 0.5% natural salicylic acid and 0.01% transdermal penetrating arginine peptide (R6).

A total of 16 subjects who met the criteria for selection and who did not meet the exclusion criteria and who were consenting to participate in the human body test were used for 6 weeks at the designated site. After 6 weeks of use, the photographs and skin measurements were taken before use and 3 weeks after use and 6 weeks after use. Subjects were asked to wash with their skin the same cleanser and after 30 minutes of stabilization in an indoor environment maintained at constant temperature and humidity.

For total lesions (non-inflammatory lesions + inflammatory lesions), the lesions were measured 3 times; after 2 weeks, after 4 weeks, and after 6 weeks. The results showed that the total lesions improved from 28.96 ± 24.25 after 2 weeks to 43.68 ± 17.97 after 4 weeks and to 51.41 ± 18.19 after 6 weeks. For total lesions, the total lesions decreased statistically, both after two weeks of use, four weeks after use and six weeks after use ($p<0.001$).

In the case of non-inflammatory lesions, five patients with less than two initial lesions could cause distortion of the results and were excluded from the statistical process and analyzed. The results represented that the total lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks. Statistically, non-inflammatory lesions decreased statistically after 4 weeks and after 6 weeks except for 2 weeks of use compared to before using acne cream ($p<0.02$).

For inflammatory lesions, the results represented that the total lesions improved from 19.08 ± 35.46 after 2 weeks to 40.00 ± 32.33 after 4 weeks and to 43.31 ± 28.49 after 6 weeks. For inflammatory lesions, the inflammatory variable decreased statistically, both after 2 weeks of use, 4 weeks after use and 6 weeks after use

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($p<0.005$).

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