

Isopropyl Myristate and Cocoa Butter are not Appropriate Positive Controls for Comedogenicity Assay in Asian Subjects

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Abstract

Background: Comedogenicity is an important consideration in development of topical products, such as cosmetics. An animal test using rabbit ear has long been served as a model for prediction of comedogenicity of topical products, but correlation to the human skin remained controversial. Isopropyl myristate and cocoa butter which are used as positive controls in animal test, also used in human clinical assay but report for the comedogenicity of those two positive controls in human, especially in Asian skin is limited.

Objective: To assess the comedogenicity of isopropyl myristate and cocoa butter in Asian skin using a modified human model established by Mill and Kligman.

Methods: We selected eight Asian subjects with prominent follicular orifices and the prone to acne type skin on the upper back. Two substances, isopropyl myristate and cocoa butter, were applied on the upper back skin three times a week for four weeks. Petrolatum and non-treated skin served as negative controls. Microcomedones were estimated by cyanoacrylate follicular biopsy at baseline and after four weeks of application. The number of follicles and microcomedones were determined by quantitative image analysis.

Results: Microcomedone activities of isopropyl myristate and cocoa butter have no significant difference to negative controls in Asian skin.

Conclusion: Comedogenic activity of isopropyl myristate and cocoa butter is too weak that those are not appropriate positive controls for clinical human assay in Asian skin. Further studies are necessary to determine appropriate positive controls in the human model for all ethnic populations.

Keywords: Human comedogenicity assay, Positive control, Asian skin, Isopropyl myristate and cocoa butter

Introduction

Comedon is a primary lesion of acne vulgaris result from abnormalities in proliferation and differentiation of keratinocytes in follicular duct of skin [1]. Comedogenic potential of a substance is an important consideration in the development of topical products such as cosmetics. Some ingredient of cosmetics having comedogenic potential could induce comedogenic symptom or worsen severity of acne condition of skin in middle-aged women during daily use of the cosmetics. The term, 'comedogenicity' and 'non-comedogenic cosmetics' are first introduced by Kligman and Mills [2] in 1972 under this concept of comedogenic potential.

An animal model using rabbit ear have long been used as a method to assess comedogenicity of topical substances [3]. Several cosmetic ingredients have been shown comedogenicity in the rabbit ear model [4-6]. Isopropyl myristate and cocoa butter served as positive controls in animal model due by its strong comedogenicity. Over the last 30 years, several human studies have been conducted under a protocol based on the rabbit ear model to evaluate the comedogenicity of topical substances using isopropyl myristate and cocoa butter as a

positive control [7,8]. However, several studies reported that the results were dissimilar from those observed in the rabbit ear model [7].

It is known that comedogenic symptoms in Asian skin are less severe compared to other ethnic populations [9,10]. Most of studies for the assessment of comedogenicity of topical substances are conducted in Caucasian skin but very few in Asian skin. Also, there are limited report from Asian skin for the comedogenicity of isopropyl myristate and cocoa butter as a positive control.

In this study, we investigate comedogenicity of isopropyl myristate and cocoa butter in Asian skin.

Materials and Methods

Eight Asian female subjects, aged between 18 and 45 years (mean age, 26.5 ± 10.2), with prominent follicular orifices and prone to acne type skin on the upper back participated in this study. Informed consent was obtained from all participants. The study protocol was approved by the ethical committee of the institute.

The casual level of sebum excretion on the center of forehead and upper back was measured by Sebumeter™ SM810 (Courage+Khazaka, Germany). Subjects were instructed not to use any topical products prior to the visit of laboratory. Measurement performed in air-conditioned room (22-24°C and 50-60% relative humidity).

Isopropyl myristate (>98% purity; Sigma Chemical Co., USA), cocoa butter (Biochemica, USA), and petrolatum (Sigma Chemical Co., USA) were prepared as supplied. 0.3 ml of each substances applied on 16 cm² area of upper back skin 3 times a week (Monday, Wednesday, and Fridays) over four consecutive weeks. Applied skin covered with a piece of non-absorbent cotton cloth (3M, USA) during 28 days of occlusive application. Patches are closely secured to the skin by Tegaderm™ occlusive and hypoallergenic adhesive tape (3M, USA). An untreated negative control skin covered by the patch materials with no substance included in all subjects. Number of follicles and microcomedones were estimated by cyanoacrylate follicular biopsy with a 3S-Biokit (CK Tech, Belgium) followed by quantitative image analysis at baseline and after four weeks of application.

The number of follicle over the number of microcomedone defined as a ratio of follicles to microcomedones. Thus the more microcomedone produced this ratio decreased. Comedogenicity expressed as a microcomedone activity, which calculated by dividing the number of microcomedones by the number of follicles. Irritation caused by repeated application of the test substances was evaluated by investigator at 30 minutes after the removal of each patch. Following scale was applied for the assessment of skin irritation; 0 (no response), 1 (slight erythema on at least 3/4 of the application area), 2 (moderate erythema or slight edema), 3 (strong erythema, slight edema), and 4 (severe response with edema, vesicles, and pustules). At the end of the study, the total irritation score of each skin area was determined from each subject by sum of individual's daily scores. Mean of total irritation score calculated.

Statistical analysis

Statistical analysis was conducted using SPSS® for Windows (SPSS Science, USA). The data are expressed as means ± standard deviation.

Test substances	Mean ratio of follicles to microcomedones		Mean value of microcomedone activity		Percentage Change of microcomedone activity from the baseline
	Baseline	4 weeks after	Baseline	4 weeks after	
Isopropyl myristate	22.50 ± 3.58	13.84 ± 1.67	0.0444	0.0723	+63%
Cocoa butter	22.20 ± 5.54	15.06 ± 0.18	0.0450	0.0664	+47%
Petrolatum	31.00 ± 11.01	19.94 ± 0.51	0.0323	0.0502	+55%
No test material	30.92 ± 9.22	20.08 ± 2.53	0.0323	0.0498	+54%

Table 2: Comedogenicity of test substances.

Test substances	Total cumulative irritation mean score
Isopropyl myristate	1.25 ± 1.49
Cocoa butter	1.75 ± 2.32
Petrolatum	0.50 ± 1.07
No test material	0.88 ± 1.13
p value	0.167

Table 3: Comparison of total cumulative irritation mean score.

The Friedman rank sum test applied to compare the difference of microcomedone activity among test substances. P<0.05 was regarded as statistically significant.

Results

Values of sebum excretion are summarized in Table 1. The mean of casual sebum level on the forehead was significantly higher than the upper back (45.71 ± 12.38 µg/cm² and 7.63 ± 11.3 µg/cm², respectively).

Clinical characteristic	Mean ± SD
Age	26.50 ± 10.16
Sebum output (µg/cm ²) i) Face	45.71 ± 12.38
ii) Back	7.63 ± 11.30
Upper back follicle density (follicles/cm ²)	35.60 ± 7.50
SD: standard deviation	

Table 1: Demographic characteristics of subjects.

Table 2 presents the mean ratio of follicles to microcomedones, microcomedone activity and the percent change of microcomedone activity. In comparison with the baseline, the microcomedone activities of isopropyl myristate, cocoa butter, petrolatum, and non-treated control increased 63%, 47%, 55%, and 54%, respectively in comparison to the baseline. There were no significant differences in microcomedone activities between test substances.

Table 3 summarizes the cumulative irritation data. All test substances showed weak responses. There were no statistical differences in adverse skin reaction potencies and irritation between two studied substances and the negative control.

Discussion

Acne vulgaris is a common skin problem in young and middle-aged people of both genders and all ethnic skin. Although, it prevails on face skin but all of the body skin, including the chest, back, and shoulders, is also subject of onset. Hormonal changes, unbalanced sebum secretion, and microbes are proved to be involved in symptomatic succession of acne vulgaris, but its pathological mechanism is not fully understood yet. Comedone is a primary lesion of acne vulgaris. It results from partial or complete obstruction of pilosebaceous duct and accumulation of sebum inside of duct. Hypercornification within the pilosebaceous duct and disturbed corneocyte dehiscence have been implicated.

Comedogenicity means a power of substance to onset comedogenic symptom on skin. Measurement of microcomedones in the rabbit ear skin or human upper back skin after topical application substances was widely used to assess comedogenicity of cosmetics or its ingredients. Isopropyl myristate and cocoa butter which proved to have a strong comedogenicity in rabbit ear model, were also widely used as positive controls in the human clinical study but correlation to human skin remains controversial.

In 1989, the American Academy of Dermatology demonstrated that, if no evidence of comedogenesis was found in the animal model, the test substance was unlikely to be comedogenic in human skin. One-plus reactions in animal model are also unlikely to cause a reaction in humans, and two-plus or three-plus responses require sound scientific judgment [11]. Mills [7] reported in his study with five young African adults that 100% isopropyl myristate was none or weak comedogenic. Then cocoa butter is obtained from whole cocoa beans which have been roasted and fermented to separate cocoa butter from their hulls. Cocoa butter contains a high proportion of fatty acids, such as stearic acid, palmitic acid, oleic acid, and myristic acid. All those fatty acids are known as comedogenic substances linked to problem of pomade acne. However, the comedogenicity of cocoa butter became controversial as its fatty acid composition varies with processing condition [12]. In our study, the comedogenicity of these two known positive controls are too weak even there was no significant difference to the negative control in Asian skin which is very similar to the results of previous study.

Several studies have compared the prevalence and characteristics of acne vulgaris among different ethnic groups. Callender et al. [13], reported that the onset of acne vulgaris in Caucasian women began earlier age than non-Caucasian women and more troublesome. Lee and Lim [9] investigated that acne vulgaris is less common among Asian than Caucasian. Other studies have shown that non-Westernized societies have a lower prevalence of acne vulgaris [9,10,14,15].

Sebum is secreted by the sebaceous glands, holocrine, multilobular glands mainly associated with hair follicles [16]. Choi et al. [17] reported that the casual level of sebum secretion closely related to the number, proportion, and location of acne vulgaris. Mangelsdorf et al. [18] found that follicular density on the forehead was significantly lower in Asian compared to Caucasian. Aramarki et al. [19] showed that casual sebum levels in Japanese women were significantly lower

than in Caucasians. Rode et al. [20] demonstrated that casual sebum levels on the forehead and back of Caucasians were about 170 $\mu\text{g}/\text{cm}^2$ and 40 $\mu\text{g}/\text{cm}^2$, respectively. In our data, sebum level of Asian skin showed 45.71 $\mu\text{g}/\text{cm}^2$ on the forehead and 7.63 $\mu\text{g}/\text{cm}^2$ on the back which were much lower than for Caucasians.

This study conducted on small number of subject but size of panel was not a critical factor for the identification of the comedogenic potential of known positive substances. Lower level of sebum secretion in Asian skin than Caucasian possibly related to not enough microcomedone activity of isopropyl myristate and cocoa butter in Asian skin but it should be investigated in further study with much more number of subjects.

In conclusion, isopropyl myristate and cocoa butter are not appropriate positive controls using the Mill and Kligman human model in Asian subjects, who have lower sensitivity to comedogenicity. Therefore, further studies are necessary to elucidate ethnic difference of the comedogenicity and to investigate appropriate positive controls in the human model for different ethnic populations.

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