A re-evaluation of the comedogenicity concept

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Background: Comedogenicity is an important consideration in the development of topical medications, cosmetics, and skin care products. The concept of "acne cosmetica" was developed to link the use of certain ingredients to comedo formation. Animal models were originally used to determine the comedogenic potential of raw materials with the assumption that finished formulations containing these ingredients would also be comedogenic. Based on this assumption, dermatologists were presented with lists of substances to avoid in patients with the ability to develop comedones.

Objective: We sought to use a modification of the Mills and Kligman human assay for assessing comedogenic potential of finished cosmetic products.

Methods: Six individuals with prominent follicular orifices and the ability to form comedones on the upper aspect of the back were enrolled. Each person received patches to the upper aspect of the back saturated with 0.2 to 0.5 mL of the finished cosmetic study products 3 times weekly for 4 weeks. Cyanoacrylate biopsies were performed to determine the number of follicles and microcomedones per square inch.

Limitations: Only a finite number of finished cosmetic products could be analyzed.

Conclusion: Finished products using comedogenic ingredients are not necessarily comedogenic. (J Am Acad Dermatol 2006;54:507-12.)

The concept of "acne cosmetica" was introduced in 1972 by Kligman and Mills[1] to link middle-aged female acne with the use of cosmetic formulations containing certain ingredients capable of producing comedones. Cosmetic researchers quickly developed animal models to predict human comedogenic activity and began to generate and publish data on various raw materials.[2-4] The rabbit ear model was conceived and various visual and histologic techniques were used to assess the presence of macrocomedones and microcomedones. Based on the rabbit ear model, it appeared that many ingredients used in cosmetics evoked a comedogenic response in animals. Ten years after the concept of acne cosmetica was described, Mills and Kligman[5] published a study exploring the effects of these chemicals in human beings and found that a human model could be used to evaluate comedogenicity; however, the results were dissimilar from those observed in the rabbit ear model.

In an attempt to standardize the methodology for comedogenicity testing, the American Academy of Dermatology held an Invitational Symposium on Comedogenicity[6] in 1989 and determined that, "If the animal model does not show evidence of comedogenesis, the test material under consideration is unlikely to be comedogenic in human skin. One-plus reactions are also unlikely to cause reactions in humans. Two-plus or three-plus responses require sound scientific judgment. Reformulation should be considered or the product should be adequately tested in humans before general use." It should be noted that the comedogenic grades used in this statement are based on a scale of 0 (no comedogenicity potential) to 3 (severe comedogenic potential).

In our study a modification of the methodology of Mills and Kligman[5] for assessing substances in a human model was used to reassess the comedogenicity of substances previously reported to be comedogenic either alone or in final formulations.

METHODS

Six individuals with prominent follicular orifices and the ability to form comedones on the upper

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Table I. Comedone formation after exposure to products containing reported comedogenic ingredients

Product	Mean ratio of follicles to microcomedones (before/after)	Mean value of follicles to microcomedones (before/after)	Change from baseline
Face powder	33.05:1/24.66:1	0.0303/0.0406	34%
Facial cleanser	32.55:1/24.46:1	0.0307/00409	33%
Facial moisturizer	31.05:1/27.37:1	0.0322/0.0365	13%
SPF-8 beach product	33.94:1/25.75:1	0.0295/0.0388	32%
SPF-25 beach product	33.83:1/27.80:1	0.0296/0.036	22%
Negative control (no test material)	34.06:1/26.66:1	0.0294/0.0375	28%
Positive control (octyl palmitate)	34.29:1/17.90:1	0.0292/0.0559	91%

SPF, Sun protection factor.

Table II. Comedone formation after exposure to products containing reported comedogenic ingredients

Product	Mean ratio of follicles to microcomedones (before/after)	Mean value of follicles to microcomedones (before/after)	Change from baseline
Facial day cream	15.11:1/13.73:1	0.0662/0.0728	10%
Facial night cream	13.10:1/11.52:1	0.0763/0.0868	14%
Facial moisturizer	12.83:1/11.97:1	0.0779/0.0835	7%
Powder makeup	13.59:1/12.60:1	0.0736/0.0794	8%
Bronzing powder	14.13:1/12.66:1	0.0708/0.0790	12%
Negative control (no test material)	14.42:1/12.86:1	0.0693/0.0778	12%
Positive control (acetylated lanolin alcohol)	15.60:1/8.37:1	0.0641/0.1195	86%

aspect of the back were enrolled in each of the two study groups. Each participant received patches to the upper aspect of the back containing a finished product with a reported comedogenic material concurrently with positive and negative controls. Patches were saturated with 0.2 to 0.5 mL of the study product, left in place for 48 hours if placed on Monday and Wednesday and 72 hours if placed on Friday, removed and visually inspected for erythema/edema, and repatched. This procedure was repeated weekly for 4 weeks. After 4 weeks, a series of 51-cm^2 follicular biopsy specimens were randomly taken from a 16-cm^2 area on the upper back. Biopsy specimens were made both prepatching (before 4 weeks of patching) and postpatching (15 minutes after last patch removal) to compare the presence of comedone formation before and after product exposure. The biopsy technique consisted of cyanoacrylate glue application to a glass slide, which was firmly placed on the intended upper back biopsy site.[7] Upon curing, the slide was rapidly removed, to maintain the integrity of the biopsy specimen. Each slide was examined microscopically to determine the number of follicles and microcomedones per square centimeter for each patch site per person. To avoid a subjective grading system, the mean ratio of follicles to microcomedones was obtained for both preapplication and postapplication sites. A percent change from baseline was calculated to determine the degree of microcomedone formation.

RESULTS

Tables I and II present data obtained for each of the two product groups evaluated. Data are expressed in terms of mean follicle to microcomedone ratio with baseline ratios compared with those obtained after 4 weeks of product application. For example, in Table I the positive control used was octyl palmitate. The mean prefollicle to microcomedone ratio was 34.29 follicles per 1 microcomedone (34.29:1). After 4 weeks of application, the mean postfollicle to microcomedone ratio changed to 17.90 follicles per 1 microcomedone (17.90:1). To determine the percent change in microcomedone formation from baseline, the ratio was converted to a numeric value by dividing the number of microcomedones by the number of follicles (1/34.29 = 0.0292 for the prevalue and 1/17.90 = 0.0559 for the postvalue). The prevalue (0.0292) was subtracted from the postvalue (0.0559), divided by the prevalue (0.0292), and multiplied by 100 to obtain the percent change from baseline of 91.44%.

This value can be used to determine the comedogenicity of a single ingredient or a final formulation. A percent change in microcomedone activity of greater or less than 10% of the negative control can

Table III. Reported comedogenic activity of various ingredients used in the products tested in Table I

Product	Problem ingredients (range used)	Histologic model comedogenic activity, scale = 0-5 (*)	Nonhistologic model comedogenic activity, scale = 0-5 (*)
Face powder	MO (25%-30%)	0-2 (100%)	0-2 (10% PG)
	Cocoa butter (<1%)	1-2 (30% MO)	4 (10% PG)
	Beeswax (1%-5%)	2 (30% MO)	0-2 (10% PG)
	Zinc oxide (>50%)	NT	1 (10% PG)
	Talc (<1%)	NT	1 (10% PG)
Facial cleanser	Cetyl alcohol (1%-5%)	0-1 (50% MO)	1 (10% PG)
	Isopropyl palmitate (1%-5%)	3 (100%)	4 (10% PG)
	Lanolin (<1%)	3 (50% MO)	3 (10% PG)
	MO (5%-10%)	0-2 (100%)	0-2 (10% PG)
	Triethanolamine (<1%)	0-2 (100%)	2 (10% PG)
	Polyethylene glycol 8 stearate (1%-5%)	NT	3 (10% PG)
	Carbomer (<1%)	NT	1 (10% PG)
Facial moisturizer	Lanolin alcohol (<1%)	1-2 (33% MO)	0-2 (10% PG)
	MO (5%-10%)	0-2 (100%)	0-2 (10% PG)
	Myristyl lactate (1%-5%)	3-4 (50% PG)	4 (10% PG)
	Cetyl alcohol (1%-5%)	0-1 (50% MO)	1 (10% PG)
	Glyceryl stearate (<1%)	2 (50% MO)	1 (10% PG)
	Stearic acid (<1%)	1-2 (50% PG)	2-3 (10% PG)
	Carbomer (<1%)	NT	1 (10% PG)
SPF-8 beach product	Isopropyl myristate (1%-5%)	3-4 (50% PG)	5 (10% PG)
	Hydrogenated castor oil (1%-5%)	NT	1 (10% PG)
SPF-25 beach product	Octyl palmitate (1%-5%)	2-3 (100%)	4 (10% PG)
	Hydrogenated castor oil (1%-5%)	NT	1 (10% PG)

MO, Mineral oil; *NT*, not tested; *PG*, propylene glycol; *SPF*, sun protection factor.
*Concentrations tested and vehicle used.

be attributed to subject variability and the test material considered noncomedogenic. Positive controls of known comedogenic compounds produce a 50% to 100% increase in microcomedone formation. Materials that produce less than a 50% increase in microcomedone activity can be considered noncomedogenic.

The data presented in Tables I and II include present the percent change in microcomedone activities for the products tested, which contain various reported comedogenic materials. These reported comedogenic values are depicted in Tables III, IV, and V.[2-4] The percent change from baseline is within the normal range of plus or minus 10% of the negative controls tested and can, therefore, be considered noncomedogenic in human beings. In addition, based on the percent change in microcomedone activity for the positive controls (octyl palmitate = 91% and acetylated lanolin alcohol = 86%) it would appear that these materials do exhibit a comedogenic response when tested undiluted in human beings. The data would appear to be paradoxical in nature because all of the products tested contain at least two ingredients previously reported to be comedogenic.

DISCUSSION

The results obtained from this study might at first appear to be confusing; however, differing results regarding comedogenicity testing are not new to the literature. Tables III and IV were compiled from previously published data to show the comedogenic activity of each of the ingredients in the formulations tested and presented in Tables I and II. It is important, however, to consider the difference between the models being compared, which are animal versus human, and the concentration at which the materials are used in the formulas representing the dose-response relationship. The main difference between the comedogenicity data generated as part of this study in Tables I and II and the literature data presented in Tables III and IV is the method used to evaluate the comedogenic response. Our study used a human model whereas the literature data were obtained using an animal model. Furthermore, there are two sets of literature data presented in Tables III and IV. There is a comedogenicity assessment based on histologic grading compared with that based on visual grading. When the evaluated animal tissue is viewed histologically, the whole follicle is assessed. When the animal is viewed visually, only follicular

Table IV. Reported comedogenic activity of various ingredients used in the products tested in Table II

Product	Problem ingredients (range used)	Histologic model comedogenic activity, scale = 0-5 (*)	Nonhistologic model comedogenic activity, scale = 0-5 (*)
Facial day cream	Butylene glycol (5%-10%)	0 (100%)	1 (10% PG)
	Beeswax (1%-5%)	2 (33% MO)	0-2 (10% PG)
	Glyceryl stearate (1%-5%)	2 (50% MO)	1 (10% PG)
	PG—Dicaprylate/dicaprate (1%-5%)	1-2 (100%)	1 (10% PG)
	Jojoba oil (<1%)	0-1 (100%)	0-2 (10% PG)
	Triethanolamine (<1%)	0-2 (100%)	2 (10% PG)
	Carbomer (<1%)	NT	1 (10% PG)
Facial night cream	Butylene glycol (10%-20%)	0 (100%)	1 (10% PG)
	Jojoba oil (1%-5%)	3 (50% MO)	3 (10% PG)
	Stearyl alcohol (<1%)	0-1 (100%)	2 (10% PG)
	Hydrogenated polyisobutene (5%-10%)	NT	1 (10% PG)
	Red 4 (1-5 parts/million)	NT	2 (10% PG)
Facial moisturizer	Triethanolamine (<1%)	0-2 (100%)	2 (10% PG)
	Carbomer (<1%)	NT	1 (10% PG)
Powder makeup	MO (10%-20%)	0-2 (100%)	0-2 (10% PG)
	Isopropyl lanolate (1%-5%)	2 (100%)	NT
	Corn oil (<1%)	0-1 (100%)	3 (10% PG)
	Zinc oxide (10%-20%)	NT	1 (10% PG)
	Talc (10%-20%)	NT	1 (10% PG)
Bronzing powder	Isopropyl isostearate (1%-5%)	4 (100%)	5 (10% PG)
	Isopropyl lanolate (1%-5%)	2 (100%)	NT

MO, Mineral oil; *NT*, not tested; *PG*, propylene glycol.
*Concentrations tested and vehicle used.

Table V. Dose-response data for reported comedogenic ingredients in the animal model

Ingredient	Concentrations	Vehicle	Comedogenic activity (histologic model)
Acetylated lanolin alcohol	2.5%	Propylene glycol	1
	50%	Propylene glycol	4-5
	100%	Not applicable	4-5
Isopropyl isostearate	5%	Mineral oil	1-2
	50%	Mineral oil	2-3
	100%	Not applicable	4
Isopropyl lanolate	50%	Propylene glycol	2
	100%	Not applicable	2
Isopropyl myristate	2.5%	Propylene glycol	0
	10%	Propylene glycol	1-3
	100%	Not applicable	2-3
Myristyl lactate	10%	Propylene glycol	2-3
	50%	Propylene glycol	3-4
Octyl palmitate	5%	Mineral oil	0
	50%	Mineral oil	1
	100%	Not applicable	2-3

dilation can be assessed. Follicular dilation can be easily misinterpreted, because dilation does not always imply follicular impaction and comedone formation. For example, materials that can evoke an irritant response, such as sodium lauryl sulfate, may dilate the follicle visually because of irritation, but have no comedogenic activity when the follicle is evaluate histologically. Furthermore, keratolytic substances, which are used to treat comedones, such as sulfur or salicylic acid, can produce follicular dilation

as the comedone is dislodged and removed from the follicle.

Other variables may also influence the results of comedogenicity testing. Table V demonstrates the effect of dose response on materials deemed comedogenic. Notice that some substances are noncomedogenic at lower concentrations and comedogenic at higher concentrations. Some dermatologists believe that if a patient is using a product that contains a comedogenic ingredient, the final product formulation must also be comedogenic regardless of concentration. In this case, it may be worthwhile to look at the packaging to see if the manufacture makes a noncomedogenic claim based on testing.

However, assessment of noncomedogenic claims can be challenging based on the findings of the current study. As pointed out previously, differing results can be obtained with different models. The comedogenicity model of choice is usually selected based on reproducibility, validity, and the ability to screen numerous materials as inexpensively and rapidly as possibly. The animal comedogenicity model demonstrates all of these attributes and can be considered an excellent screen, but not necessarily the end point for final determination. The controversial aspect of any model is definition of the end point. In the case of comedogenicity testing, it is the number of application (5, 10, or 15), timing of applications (once or twice/d), amount applied (0.1 or 0.5 mL), duration of applications (1, 2, or 3 times/wk), method of response evaluation (visual or histologic), and evaluation scale (0-3, 0-4, 0-5). These variables must be considered when evaluating the reported comedogenic potential of individual ingredients or final formulations.

It is important to recognize that the human model has strengths and weaknesses, as does the animal model for comedogenicity assessment. The human model uses exaggerated conditions to extrapolate the results to a broad population. First, the test materials are applied under occlusion to the back for 4 weeks opposed to topical facial application in actual use. This enhances product absorption and penetration, and the ability to evoke microcomedone formation. Second, panelist selection requires the presence of prominent follicular orifices indicating the propensity to form microcomedones. Third, this is but one of many tests used by reputable manufacturers to determine safety. Most combine comedogenicity testing with a clinical use test whereby individuals use the products under normal conditions of use for several weeks carefully noting the presence of comedone and acne formation.

During the last 10 years since the human comedogenicity model has been used, the ingredients used as positive controls, mainly acetylated lanolin alcohol and isopropyl myristate, have repeatedly produced 75% to 100% increases in microcomedone counts from baseline. Yet, there remains considerable variability between individuals with respect to the number of follicles and microcomedones per square centimeter. Notice that in Tables I and II the premean follicle to microcomedone ratio for product group I ranged from 31.05:1 to 34.29:1 whereas the similar ratio for product group II ranged from 12.83:1 to 15.60:1. This observation points out the importance of using a positive and negative control concurrently with the products tested. Thus, the determination of comedogenicity should be based on percent change from baseline in microcomedone counts. The value of expressing the result as a percentage, as opposed to a comedogenic grade (eg, 0, 1, 2), controls for the intragroup subject variability between groups. For example, in our study, the preratios for the positive controls used in the two different groups were 34.29:1 for octyl palmitate versus 15.60:1 for acetylated lanolin alcohol, which are quite different. Yet, the percent change from baseline was similar at 91% for octyl palmitate and 86% for acetylated lanolin alcohol. Even greater intragroup variability was seen between the negative control groups. Note that the percent change in microcomedone formation from baseline for group I was 28% versus 12% for group II. This is primarily a result of comedogenicity measured at the low end of the scale and further depicts subject variability.

It is important to note that the original data published by Morris and Kwan[3] and Fulton[4] regarding comedogenicity using animals relied on the external ear canal of only 3 rabbits. The method used to assess the formation of comedones was open application of the materials. Our study used 6 individuals who underwent thrice-weekly application of the materials under occlusive dressing, which enhances penetration. Even though this sample size may seem small, a large comedogenicity study is considered 10 subjects in the skin care and cosmetics industry.

This study has evaluated some of the controversies regarding comedogenicity. This concept remains important to the dermatologist in terms of product safety and performance. The skin care and cosmetics industry has developed a variety of models and assessment criteria to predict the comedogenicity of single ingredients and final formulations. As of this writing, comedogenicity assessment remains an art, similar to many aspects of dermatologic practice.

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